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INTRANUCLEAR AND CYTOPLASMIC INCLUSIONS ("PROTOZOAN-LIKE BODIES") IN THE SALIVARY GLANDS AND OTHER ORGANS OF INFANTS *

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A routine study of the salivary glands in a fairly large series of postmortem examinations on infants has revealed a hitherto unsuspected large number showing intranuclear and cytoplasmic inclusions in the duct epithelium, often in intimate association with foci of lymphocytic infiltration. In two infants inclusion bodies were found in cells in epithelial-lined spaces in various organs of the body. These findings are included in this report.

Literature

Ribbert [1] in 1881 first noted large "protozoan-like" cells in the kidney of a luetic stillborn infant. In 1904 he published this observation, together with a description of similar structures in the parotid glands of two non-luetic infants. The large cells occurred within ducts, singly or in groups. He was preceded in publication by Jesionek and Kiolemenoglou,[2] who noted the large cells in the lungs, kidneys and liver of an 8 month luetic fetus. The large cells averaged from 20 to 30 microns in diameter and were usually oval in outline with a well defined, though not sharply stained, cuticular zone having the appearance of a capsule. The nuclei were large and eccentrically placed. Each contained a "central nuclear body" surrounded by two well defined zones, an inner dark and an outer clear zone. In the clear zone deeply staining granules averaging

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1 micron in diameter were found. The cell body was spongy and the pole near the nucleus was filled with granules. The nucleus appeared to be separated from the cell body by a membrane. The large cells were most numerous in the interstitial tissues of the kidney, in association with areas of congenital luetic inflammation.

Löwenstein,[3] working in Ribbert's laboratory in 1907, found inclusions in both parotid glands in four of thirty infants. He described without recognition the cytoplasmic, as well as the nuclear inclusions. Additional instances were reported in 1910 by Pisanò,[4] who found inclusions in the kidneys, liver and lung, and Mouchet,[5] who noted inclusions in the bile ducts. Pettavel[6] in 1911 studied the thyroid of a 10 day old prematurely born infant, and described peculiar degenerative changes in the epithelial cells. Although he did not recognize the inclusions, the illustrations in his paper leave no doubt that he was dealing with the same type of inclusion bodies. This marked the first finding of the inclusions in the thyroid gland. In 1910, and again in 1914 Smith and Weidman[7, 8] described similar findings and concluded that they were dealing with protozoa. They gave the name *Endameba mortinatalium* to the structures. Jackson[9] in 1920 called attention to cells which she called protozoan parasites, in the ducts of salivary glands of guinea pigs. These were apparently identical with those noted in infants. Goodpasture and Talbot[10] in the following year reported the finding of similar structures in the lungs, liver and kidneys of a 2 months' old infant. The salivary glands were not examined. On the basis of their study of this case, and of salivary glands of guinea pigs, they concluded that they were dealing with a new kind of abnormal cytomorphosis, to which they gave the name *cytomegalia*. They stated definitely that the structures were not protozoa and they described not only the intranuclear inclusions, but the cytoplasmic inclusions as well.

In the following year de Lange[11] reported the inclusions in the kidney, and Müller[12] described a similar finding in the kidneys of three infants. VonGlahn and Pappenheimer[13] found the inclusions in the intestines, liver and lungs of a 36 year old man, for the first time in an adult. Walz[14] in 1926 observed the inclusions in the pancreas, as well as in the kidneys, liver, lungs and thyroid of a newborn infant. In a discussion of Walz's paper von Albertini mentioned a similar unreported observation. The last case report was by Wagner,[15] who noted the inclusions in the lungs, kidneys, liver,

pancreas, thyroid, epididymis and sublingual gland of a 2 weeks' premature infant, in whom no evidence of congenital lues could be found. He also found the inclusions in the parotid glands of four of a small series of infants.

An excellent review of the cases mentioned above, and a discussion of the various explanations advanced are given by VonGlahn and Pappenheimer,[13] so that a more complete review need not be given here. The cases are summarized in Table I, which is a combination of the tables of VonGlahn and Pappenheimer,[13] and Walz,[14] with corrections and additions. It will be noted that the distribution of the inclusions in the various organs of the reported instances is as follows:

Kidneys	11 cases
Parotids	10
Lungs	8
Liver	8
Pancreas	2
Thyroid	3
Intestine	1
Sublingual gland	1
Epididymis	1

Goodpasture and Talbot [10] first called attention to the similarity of these bodies to a structural variation in the intranuclear body described by Tyzzer [16] in cutaneous lesions in varicella. Although the protozoa theory was kept alive in Germany until 1930 (Wagner [15]) a new significance was given these findings in 1921, when Lipschütz [17] reported that similar structures are constantly associated with the lesions of the herpes virus in man and rabbits. Later, due to the work of Cole and Kuttner [18, 19] and to a number of intensive studies which have appeared from the laboratories of Goodpasture [20, 21] and Cowdry [22, 23] and their associates, a mass of data has accumulated to show that a definite relation does exist between inclusion bodies and certain types of filtrable virus disease (variola, vaccinia, sheep-pox, fowl-pox, molluscum contagiosum, herpes, submaxillary virus disease of guinea pigs, and so on). Goodpasture [20] believes that such intranuclear inclusions indicate an intranuclear localization of the infective substance in filtrable virus disease. The controversial theories and data are admirably expressed by Goodpasture [20] in a recent review of the subject of inclusion bodies in relation to the etiology of virus diseases.

TABLE I

Reported Cases with Inclusion Bodies

No.	Year	Author	Age	Location of Inclusions									Pathological diagnosis	Interpretation
				Kidney	Parotid	Lung	Liver	Pancreas	Thyroid	Intestine	Sublingual gland	Epididymis		
1	1904	Jesionek and Kiolemenoglou	Stillborn	×	..	×	×	..	..	..	..	..	Congenital lues	Gregarines (R. Hertwig)
2	1904	Ribbert	Stillborn	×	..	..	..	..	..	..	..	..	Congenital lues	Amebae or sporozoa (Ehlers-Rhumbler)
3			1 yr.	..	×	..	..	..	..	..	..	..	No lues	
4			3 mo.	..	×	..	..	..	..	..	..	..	No lues	
5	1907	Löwenstein	2 mo.	..	×	..	..	..	..	..	..	..	....	Coccidia (Ludwig)
6			3 mo.	..	×	..	..	..	..	..	..	..	....	
7			10 mo.	..	×	..	..	..	..	..	..	..	....	
8			3 mo.	..	×	..	..	..	..	..	..	..	....	
9	1910	Mouchet	8 days	..	..	..	×	..	..	..	..	..	Congenital lues	Sporozoa
10	1910	Pisanò	Stillborn	×	..	×	×	..	..	..	..	..	Congenital lues	Embryonic epithelial cells
11	1910	Smith and Weidman	Stillborn	×	..	×	×	..	..	..	..	..	Focal nephritis	Endameba mortinatalium
12	1911	Pettavel	10 days	..	..	..	..	..	×	..	..	..	Purpura	Peculiar epithelial degeneration

13	1914	Smith and Weidman	2 mo.	..	..	×	..	..	..	..	..	..	Pneumonia	Endameba mortinatalium
14		Goodpasture and Talbot	6 wks.	×	..	×	×	..	..	..	..	..	Edema, cough and loss of appetite	Abnormal cytomorphosis "cytomegalia"
15	1922	de Lange	8 days	×	..	..	..	..	..	..	..	..	Congenital lues?	Cellular degeneration
16	1922	Müller	8 wks.	×	..	..	..	..	..	..	..	..	Hydrocephalus, nephritis	Degeneration
17			Stillborn	×	..	..	..	..	..	..	..	..		
18			2 mo.	×	..	..	..	..	..	..	..	..	Lues	
19	1925	VonGlahn and Pappenheimer	36 yrs.	..	..	×	×	..	..	×	..	..	Abscess of liver, ulcerative colitis, pneumonia	Filtrable virus Inclusion bodies
20	1926	Walz	20 min.	×	..	×	×	×	×	..	..	..	Prematurity, asphyxia	Protozoa
21	1930	Wagner	2 wks.	×	..	×	×	×	×	..	×	×	Prematurity	Undecided
22			up to 2 yrs. of age	..	×	..	..	..	..	..	..	..		
23				..	×	..	..	..	..	..	..	..		
24				..	×	..	..	..	..	..	..	..		
25				..	×	..	..	..	..	..	..	..		
	Total No. cases			11	10	8	8	2	3	1	1	1		

× = present

Pearson [24] in a recent study called particular attention to the cytoplasmic inclusions. In a study of guinea pig salivary glands he found these inclusions to be spherical or oval in shape, and varying in size from a fraction of a micron up to 6 to 8 microns. They do not contain fat or lipoid in demonstrable amounts. Pearson had the opportunity of studying two cases from our group reported here, and found the guinea pig cytoplasmic inclusions indistinguishable from "certain cytoplasmic inclusions of rare occurrence in the human submaxillary glands." We have examined the salivary glands of a small number of normal guinea pigs and have noted on several occasions inclusions apparently identical with those found in our series of infant submaxillary glands. The guinea pig salivary gland inclusions were often accompanied by marked lymphoid infiltration.

In a discussion to a preliminary report of this study [25] Dr. Oskar Klotz of Toronto mentioned that inclusions similar to those described here were found by Dr. J. Thompson in the submaxillary glands in 14 per cent of a series of rats 2 months' old. These rats had been subjected to experiments on vitamin D over a short period. On the same occasion, Dr. E. V. Cowdry of St. Louis stated that Dr. G. H. Scott had found no inclusions in one hundred newborn infants and fetuses collected in St. Louis, Minneapolis and the Middle West.

Discussion of Present Series

The submaxillary glands, and often the parotid glands, were removed in a consecutive series of autopsies on infants to determine the incidence of inclusion bodies in the salivary glands of infants, and to study the clinical and general pathological features of the cases in which these inclusions occurred. A portion of each gland removed was put immediately into sterile glycerine for further experimental work, and the remainder fixed in Regaud's fluid. Routine stains were made with hematoxylin and eosin; Giemsa and eosin-methyline blue were also employed. A study of the preparations showed the inclusions in twenty-two or 12 per cent of the 183 cases studied. In addition, two cases in which the submaxillary inclusions were noted on a previous occasion, and two others in which inclusions were found in various body organs were studied with this series, making a total of twenty-six cases available for clinical and general pathological analysis. It might be well to stress

the fact that in none of these cases was attention called to the submaxillary or parotid glands during life.

The clinical and pathological records of the twenty-six patients can be summarized as follows:

Age: Twenty-one instances ranged from 2 days to 1 year of age; the remaining five from 13 months to 17 months.

Sex: There were fifteen males and eleven females.

Season: The cases were scattered throughout the period of a year, with no definite seasonal preponderance.

Past History: In twenty-five of the twenty-six infants, the past history was essentially negative. There was no history of mumps or of infection in the general region of the salivary glands in any of the cases. The group includes both breast and artificially fed infants. Only one of the group had a history of contagious disease. This patient had measles, followed four months later by fatal miliary tuberculosis at 17 months' of age.

Present Illness: Vomiting and diarrhea marked the onset of illness in four cases. These were regarded as acute nutritional disturbances. In three instances the fatal illness had a sudden onset and a brief course, with death occurring in five to twenty hours. One of these had *Streptococcus hemolyticus* septicemia, one acute fulminating meningococcus meningitis, and the third died five hours after an operation for the repair of a large umbilical hernia.

There were symptoms referable to the central nervous system in several cases of meningitis due to various microörganisms, but there were no cases with unexplained manifestations of central nervous system disturbances. The remainder of the group had signs and symptoms referable to acute inflammation somewhere in the body, most often in association with the upper respiratory tract.

Clinical Course: The clinical course was variable and was usually characteristic of the particular disease. The duration of the fatal illness varied from several weeks to several hours.

Temperature: The temperature was usually high, varying in most cases from 101° to 104° F, the highest temperature occurring terminally in the instances of tuberculous meningitis.

Cause of Death: An adequate cause of death was found in every case. Five died of acute miliary tuberculosis, one having in addition a *Streptococcus hemolyticus* septicemia. Three could be grouped under the term "acute nutritional disturbance" ending with terminal infections. Bronchopneumonia and otitis media were the main features in three instances. There were two cases of pneumococcus septicemia, and two (not of the present series) of keratomalacia. Congenital lues occurred in but two patients. The other causes of death occurred singly (Tables II and III).

This summary indicates that there are no findings which would justify the grouping of these cases into a single, or even a homogeneous, clinical or pathological class. The outstanding features common to most of the group are hyperpyrexia and acute infection somewhere in the body. The occurrence of such heterogenous con-

TABLE II

Present Series with Inclusions in Submaxillary Glands

No.	Case No.	Age	Sex	Pathological diagnoses
1	A-29-245	10 mo.	Male	Miliary tuberculosis, Strep. hem. septicemia
2	A-29-246	11 mo.	Female	Idiopathic hypertrophy of heart, terminal pneumonia
3	A-30-1	8 mo.	Male	Congenital lues, Strep. hem. septicemia
4	A-30-8	12 mo.	Female	Miliary tuberculosis, tuberculous meningitis, osteomyelitis
5	A-30-39	7 mo.	Male	Pneumococcus septicemia, pneumonia, otitis media
6	A-30-52	8 mo.	Male	Strep. hem. septicemia
7	A-30-69	17 mo.	Male	Miliary tuberculosis
8	A-30-72	5 mo.	Female	Pneumonia, enteritis
9	A-30-78	17 mo.	Female	Pneumonia, otitis media
10	A-30-79	15 mo.	Female	Umbilical hernia, pneumonia
11	A-30-80	17 mo.	Female	Miliary tuberculosis, pneumonia
12	A-30-84	3 mo.	Male	Pneumonia, otitis media
13	A-30-98	11 mo.	Female	Meningococcus meningitis
14	A-30-99	7½ mo.	Female	Strep. hem. septicemia, pneumonia
15	A-30-107	3 mo.	Female	Pneumonia, otitis media
16	A-30-110	8 mo.	Male	Pneumonia, otitis media
17	A-30-111	7 mo.	Female	Meningococcus meningitis
18	A-30-117	13 mo.	Male	Miliary tuberculosis
19	A-30-181	2½ mo.	Male	Pneumonia, otitis media
20	A-30-192	6 mo.	Male	Pneumonia, otitis media
21	A-30-242	8 mo.	Male	Chronic bronchopneumonia
22	A-31-13	3½ mo.	Male	Congenital lues, pneumococcus septicemia
23	A-1009	5 mo.	Male	Keratomalacia
24	A-24-48	4½ mo.	Male	Keratomalacia

TABLE III

Present Series with Inclusions in Viscera

No.	Case No.	Age	Sex	Pathological diagnoses
1	A-30-159	20 days	Female	Hemorrhagic diathesis, inclusions in lungs, kidneys, liver, pancreas and thyroid
2	A-31-110	2 days	Male	Erythroblastosis, inclusions in kidneys, lungs, pancreas and liver

ditions in even so small a series can serve to halt, for a time at least, any speculation as to the association of the inclusion bodies with any single disease.

The fact that 80 per cent of the cases occurred in individuals under 1 year of age, that is, during a period when known diseases associated with a filtrable virus are rare, is of more than passing

interest. The series is naturally too small to permit any conclusions in regard to sex or seasonal incidence. The past history was essentially negative, except in one instance where measles occurred four months before death. Local factors are ruled out by the absence of a history of mumps or of any apparent lesion of the salivary glands during life. The clinical signs and symptoms were all satisfactorily explained by the clinical course and the postmortem findings. Of particular interest is the fact that there were no unexplained central nervous system disturbances. Congenital lues, with which many of the early reported cases were associated, was found but twice in this series. A listing of the cases by diseases and causes of death would show, in general, an approximate cross-section picture of the entire series from which this group was selected.

Gordon[26, 27] in 1913, and again in 1914 reported the association of pyrexia, collapse, diarrhea and vomiting, with symptoms of meningeal irritation followed by death in from twenty-four hours to twelve days in a group of twelve children, who at autopsy showed no adequate cause for the signs and symptoms. In the salivary glands of these patients Gordon found areas of interstitial inflammation consisting mainly of lymphocytes. His descriptions and illustrations resemble very much similar areas of infiltration which were found in the vicinity of the inclusions in our material. However, Gordon states definitely that the ducts were clear, and he makes no mention of either intranuclear or cytoplasmic inclusions. He examined the salivary glands of thirty other individuals, varying from infancy to old age, and found two of that group which showed areas of lymphocytic infiltration similar to those found in his main group. These were both adults who died of peritonitis. There are no points of similarity between Gordon's group and the present series, except clinically the hyperpyrexia, and pathologically the areas of lymphocytic infiltration in the salivary glands.

Description of Microscopic Findings

In the series of submaxillary glands studied, the number of cells containing inclusion bodies varied from large collections scattered over many fields to single cells which were found only after a long search. Often inclusions were present in one gland only. When two blocks were taken from the same gland the inclusions were some-

times absent in one. Where the inclusions were numerous the ducts were often dilated, and areas of lymphoid infiltration (Fig. 1) were usually present in the immediate vicinity of the inclusion-laden ducts and acini, replacing areas of gland parenchyma. Such areas of infiltration are similar to those described by Gordon and were the most prominent accompanying pathological processes. In a few of the "negative" gland preparations areas of acute inflammation consisting of collections of polymorphonuclear leucocytes were noted, but inclusions were lacking. Where the inclusions were rare lymphocytic infiltration was usually absent, and there was no demonstrable associated pathological process. The large cells were found always in acini and ducts of the submaxillary glands, and their relationship to the lining epithelial cells appeared definite.

In the viscera the cells were always in epithelial-lined spaces — in the tubules of the kidney, the bile ducts of the liver, the acini and ducts of the pancreas, the acini of the thyroid, and the alveoli and bronchioles of the lung. They were never found free in the interstitial tissues, blood vessels, or in association with cells of other than epithelial type. This is in contrast to the observations of several of the earlier authors. No distinctive pathological process was found in these organs, the large cells often being found in otherwise normal appearing areas. In one kidney tubule there were large cells, so numerous and large in size that the lumen of the tubule appeared almost obliterated. The greatest number of large cells found in the organs of the body were in the kidney, lung and liver. Relatively few large cells were found in the pancreas and thyroid. None were noted in the intestine (VonGlahn and Pappenheimer). The epididymis was unfortunately not examined, so the observation of Wagner could not be verified.

The cells varied greatly in size, most authors mentioning a variation of from 10 to 35 microns, with an average size of 25 microns. Most often the large cells could be recognized under low powers, after some training. The shape of the cells varied from round or oval to elongated or markedly irregular outlines. Rare multinucleated cells containing inclusions were found. A sharp nuclear membrane divided the nucleus from the cytoplasm. Within the nuclear membrane was a large inclusion body which varied considerably in size, shape and staining intensity. Usually the intranuclear inclusion appeared as an ovoid or elongated, dense, homogeneous

acidophilic body. Often the body stained more deeply in the central portion and shaded off slightly to a paler portion at the periphery. The outline of the body was usually not sharp. In some instances delicate honeycombing to coarse vacuolization could be observed within the inclusion body. No finer structures could be recognized. Surrounding this body, and between it and the nuclear membrane, there was usually a clear zone which varied in size with the outline of the nuclear inclusion body. Occasionally the clear zone was entirely obliterated by the encroachment of a vacuolated, swollen, nuclear inclusion body. In the clear zone there were usually one or two, sometimes three or four small, round to oval or spherical, dense, basophilic, granular masses, which in some cases had apparently fused to form irregularly shaped, densely staining clumps. In rare instances, in the cells of comparatively small size, no definite nuclear body was found. In the clear zone of such nuclei twenty to thirty small, densely staining masses of chromatin material were scattered, sometimes distributed in almost concentric arrangement, and at other times gathered in groups adjacent to the nuclear membrane. This we regard as an early stage in the formation of the inclusions. One perplexing feature in our study of the inclusion bodies was the failure to find small forms which could with confidence be interpreted as stages in formation. If the inclusions were present at all they were strikingly alike and within a constant narrow range of size and detail.

The cytoplasm of the cells with inclusions was basophilic in staining reaction, and usually contained a few to large numbers of dense, basophilic, oval to spherical granules which varied greatly in size. Often these cytoplasmic inclusions appeared almost round in shape, and were arranged in curved rows, conforming to the shape of the cytoplasm. The cytoplasmic inclusions were present in almost all of the large cells in the submaxillary glands, and in most of the large cells in the organs of the body. Often, when they were apparently lacking, better stained sections would bring the cytoplasmic inclusions out more clearly. The cytoplasmic inclusions do not represent mucus droplets, as might be at first suspected. Microchemical studies were carried out by Pearson,[24] who found that specific tests for mucin yielded rather inconclusive results. Furthermore, we have repeatedly observed cytoplasmic inclusions in epithelial cells lining structures where mucus-secreting cells normally do not occur.

Summary and Conclusions

In the submaxillary glands removed in a series of 183 postmortem examinations on infants, large cells containing intranuclear and cytoplasmic inclusion bodies ("protozoan-like bodies") were found in twenty-two cases (12 per cent). In addition, two older cases with inclusions in the parotid and submaxillary glands, and two instances in which inclusions were found in epithelial-lined spaces of the liver, lungs, kidneys, pancreas and thyroid are reported, making a total of twenty-six new cases to be added to the twenty-five already in the literature. All the patients in this series were less than 17 months of age, the majority being under 1 year. These inclusions are apparently identical with those found in the submaxillary glands of guinea pigs, and are generally similar to inclusions which are found in diseases due to filtrable viruses. Clinical and pathological studies of the series reported reveal no association with any distinctive feature or group of symptoms or disease changes. The frequency of the inclusions in our postmortem series suggests geographical factors affecting this occurrence and leads naturally to the suspicion of the existence of a disease in infants of filtrable virus etiology. However, if that be true, there are no distinctive clinical or pathological features which would permit its recognition on the wards or in the pathology laboratory. The clinical and pathological findings in the "positive" instances resemble, in general, the findings in the entire group studied.

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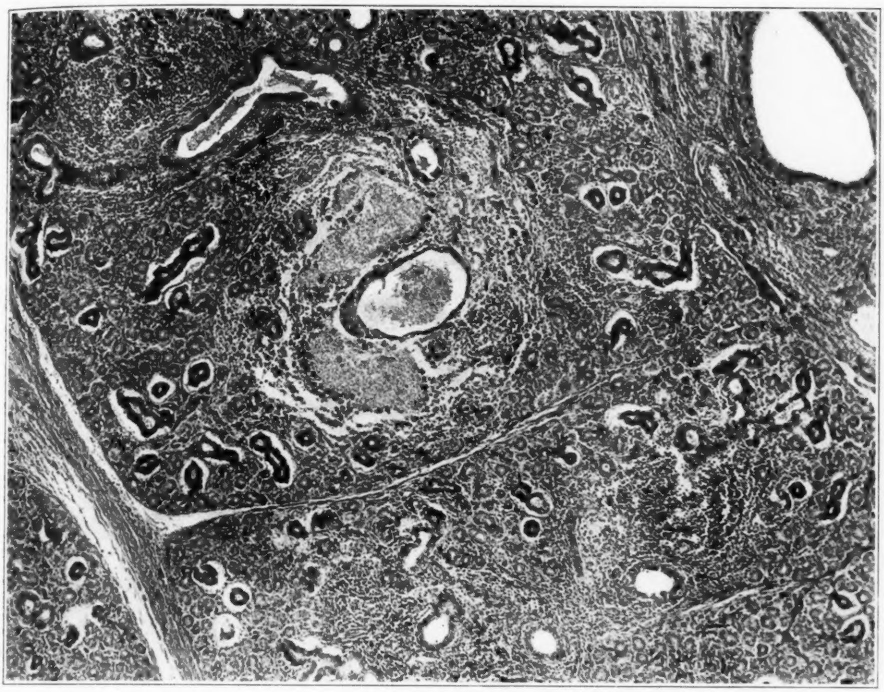
DESCRIPTION OF PLATES

Plate 22

Fig. 1. Photomicrograph of submaxillary gland. Note areas of lymphoid infiltration. Hematoxylin and eosin. Low power.

Fig. 2. Photomicrograph of submaxillary gland showing large cells with inclusions lining the duct. Hematoxylin and eosin. × 550.



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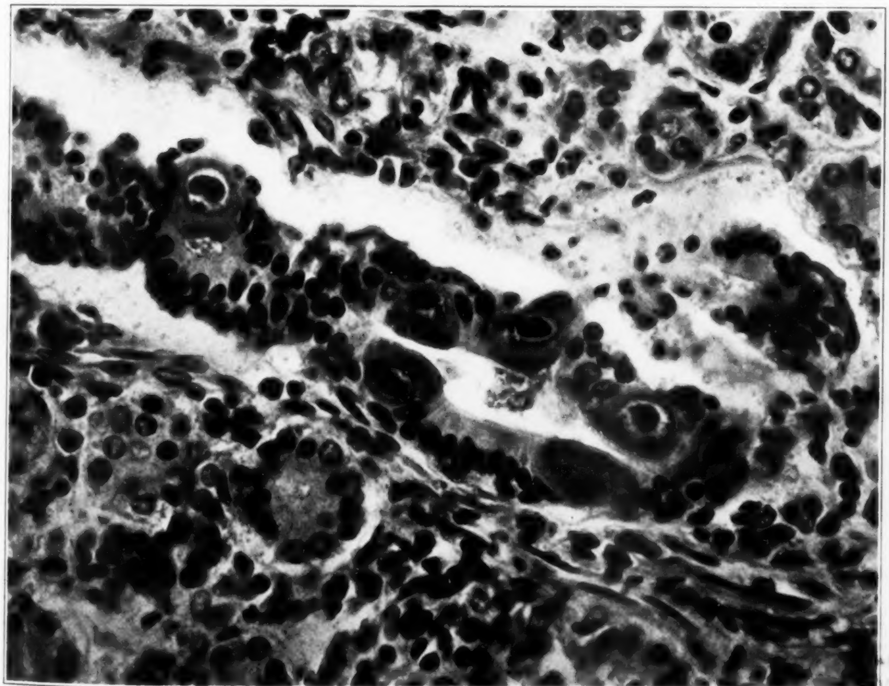

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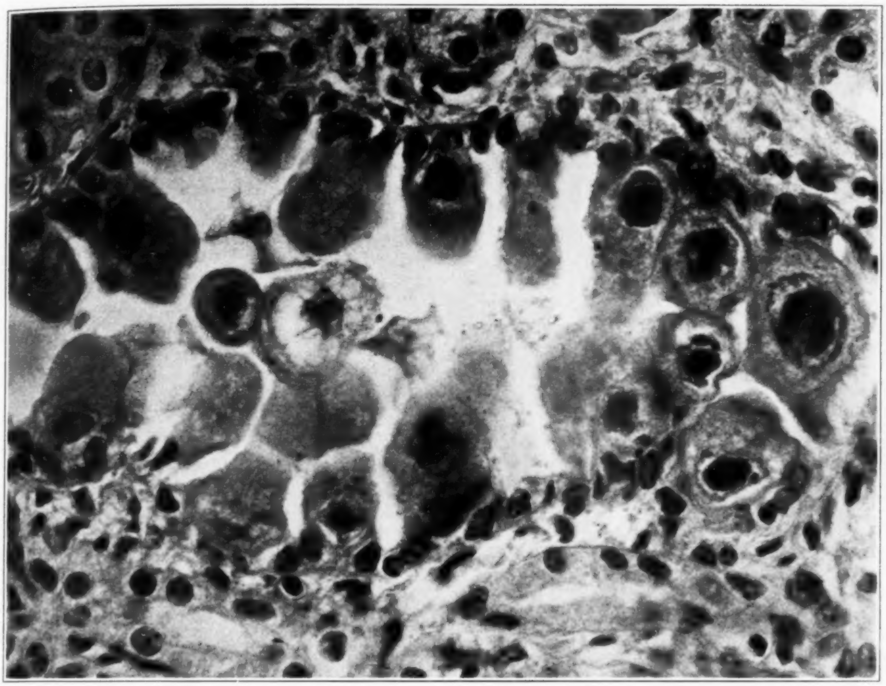
Plate 23

Fig. 3. Photomicrograph of kidney showing large inclusion-laden cells lining the kidney tubule. Note the swollen cytoplasm and vacuolated appearance of some of the cells. Hematoxylin and eosin. × 750.

Fig. 4. Photomicrograph of submaxillary gland showing a large cell in the duct lining. Note intranuclear inclusion, pale area at periphery, clear zone, small masses in clear zone, nuclear membrane and large inclusion bodies in cytoplasm. Hematoxylin and eosin. × 2300.



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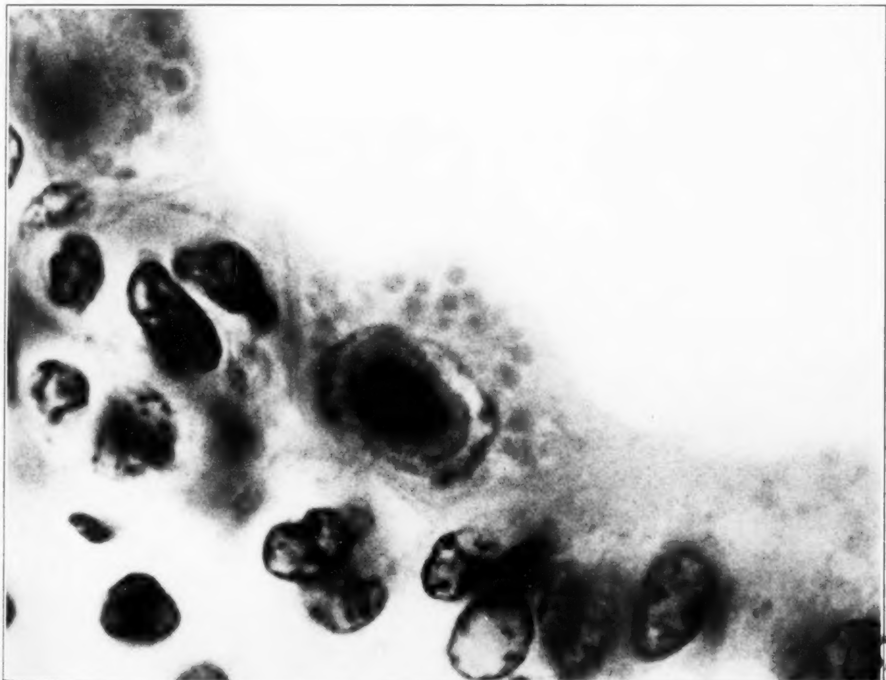

4

YELLOW FEVER ENCEPHALITIS OF THE MONKEY (MACACUS RHESUS) *

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Since the discovery by the American Commission at Havana in 1900 that the etiological agent of yellow fever is filterable, this infection has generally been grouped tentatively with the virus diseases. In later years this classification has seemed more insecure because it has become apparent that certain bacteria, protozoa and spirochetes may pass through similar filters. The finding of leptospiras by Noguchi in a group of cases clinically diagnosed yellow fever made it seem for a while still less likely that the causative agent belongs to the virus group.

Recently, however, a mass of evidence has been gathered which seems to place the active agent of yellow fever not only among the filterable viruses, but also with the cytotropic group of these infectious agencies. The recent rapid accumulation of this evidence resulted directly from the discovery by Stokes, Bauer and Hudson [1] that yellow fever may be transmitted with regularity to the Indian monkey, *Macacus rhesus*. With this animal available for the experimental study of the disease many important facts have come to light.

Bearing upon the viral etiology of yellow fever was the absence of leptospiral infection in the West African cases, the reconfirmation of the filterability of the active agent in the blood stream, and the discovery by Torres [2] that intranuclear inclusions are to be found in the injured cells of the liver of experimentally infected monkeys. Shortly afterward similar inclusions were described by Cowdry and Kitchen [3] in liver cells of human cases of West African yellow fever.

In commenting upon these important discoveries I recently expressed doubt that the agent of yellow fever should be classified as a cytotropic virus, even though these two basic facts of filterability and specific cellular inclusions were available, for the reason that

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cytotropic filterable viruses should be known to reproduce themselves locally in association with the presence of cellular inclusions.[4] This evidence was lacking, inasmuch as the inclusions had been found only in the liver cells, and there seemed to be no evidence that virus was regenerated locally in the liver in association with them.

This essential condition seems now to have been satisfied through the extraordinary discovery by Theiler [5] that the virus of yellow fever appears to be infectious for the brain of mice; and his experiments indicate it may be successfully cultivated in series in this tissue, with the induction of a fatal encephalitis. Theiler's observations further show that the mouse encephalitic virus is essentially restricted in its distribution in fatal infections to nervous tissue and adrenal gland, which has a considerable nervous component in its medulla. The virus, according to his observations, also passes centrifugally from the spinal cord along peripheral nerves. These facts definitely relate the mouse strain of virus to other neurocytotropic viruses, namely those of rabies, poliomyelitis, enzoötic encephalomyelitis and herpex simplex.[6]

Furthermore, the recent investigations of Sellards [7] contribute the important information that the mouse virus passed serially through brains of mice becomes modified in its action upon monkeys (*M. rhesus*), in that it will then induce a fatal encephalitis in these animals when inoculated intracerebrally, without causing the usual symptoms and hepatic changes of the original yellow fever virus.

Theiler was rightly cautious in his attitude toward the question of the identity of the mouse encephalitic virus and that of yellow fever, notwithstanding the fact that inclusions quite similar to those found in the liver in the natural and experimental disease were to be observed in the central nervous system of the infected mice, and, what is more significant, that yellow fever immune serum from both monkey and man showed protective power for mice inoculated with the encephalitic virus.

Evidence of the identity of the mouse virus and that of yellow fever is further contributed by the investigations of Sellards, who showed that monkeys can be immunized against typical yellow fever virus by intraperitoneal injections of mouse virus, and that monkeys immunized to typical yellow fever virus manifest a well marked, though not entirely complete protection against intracerebral injection of virulent mouse virus. Sellards concludes that the results

of these cross-immunity tests are entirely consistent with the interpretation that the virus in mice is that of yellow fever, and there is no indication that it is contaminated by any secondary virus. He states however that the amount of data available at present is not overwhelming and there is no urgent need for drawing any altogether final conclusions. In regard to hepatic lesions in monkeys infected intracerebrally with mouse virus he states: "Of five normal monkeys injected into the brain with mouse virus, none showed lesions of the liver comparable to the changes which occur in man or in monkeys dying of typical yellow fever. In one of these monkeys, a moderate amount of necrosis of the liver was found, and in another, the liver was normal. In the remaining three monkeys the liver showed moderate degenerative changes consistent with the earlier changes seen in yellow fever but by no means diagnostic and quite unlike the extensive necrosis seen in monkeys dying in the usual manner."

For a more detailed study of the cytology and histology of mouse virus encephalitis in mice and monkeys, Dr. Sellards kindly sent to me stained sections and blocks of tissue from mice and monkeys infected with this virus, and in addition, for comparison, sections of human livers from West African cases of yellow fever, and of livers from monkeys experimentally infected with yellow fever virus. This report is based entirely upon a study of the material which Dr. Sellards made available to me for this purpose.

In addition to the sections of human and monkey livers infected with yellow fever and sections and tissue from the brains of encephalitic mice, there was material from the following groups of experiments upon monkeys.

Group I: Included tissue from two monkeys which had received intracerebral injections of the typical monkey strain of yellow fever virus.

Group II: Included tissue from five normal monkeys inoculated intracerebrally with encephalitic virus from mouse brains.

Group III: Included tissue from four normal monkeys inoculated intracerebrally in series with virus originating from a monkey dead of mouse virus encephalitis.

In sections from the livers of one human and two monkeys, stained with methylene blue and eosin, intranuclear inclusions were found which correspond in all respects to the description of yellow fever

inclusions depicted by Torres and by Cowdry and Kitchen. The impressions gained from a study of these preparations served as a guide to the study of the encephalitic lesions.

Sections of brain from the two monkeys in Group I, which received typical yellow fever virus intracerebrally, show no evidence of encephalitis or of intranuclear inclusions. The livers of both animals contain the typical necrosis of yellow fever infection. As pointed out by Sellards, the typical yellow fever virus, even though introduced directly into the substance of the brain, brings about the usual appearance of yellow fever uncomplicated by a viral encephalitis.

Sections of the brains of mice dead of encephalitis show, as first described by Theiler, a perivascular mononuclear cellular exudate particularly marked in my preparations in the basal ganglia. In the brain of a young mouse, dead six days after inoculation, abundant nuclear inclusions were observed both in the ganglion cells of the cerebrum and those of the basal ganglia. It was noted that extensive necrosis of ganglion cells accompanied the presence of inclusions, and this without any evidence of cellular exudate. It seems evident from a study, both of the encephalitis of mice and of monkeys, that, as in other neurocytotropic virus lesions, the first change is in the ganglion cells, and inflammatory exudate is secondary, apparently to cellular necrosis. In an adult mouse brain perivascular infiltration and focal inflammatory exudate are prominent, but only a few inclusions were observed. This single observation suggests, on cytological grounds, that the brains of young mice are more susceptible to the virus.

The intranuclear inclusions observed in the brains of mice correspond in appearance in every way to the now well known descriptions of Torres and of Cowdry and Kitchen. Following is a description of lesions found in the brain of two mice, the first a baby mouse dead on the sixth day after inoculation, the second an adult mouse.

Baby Mouse — 6th Day

Cerebral Cortex and Basal Ganglia: (Stained with methylene blue and eosin.) Changes in ganglion cells throughout the sections are to be seen in great abundance. They are more numerous in the basal ganglia, but are also diffusely scattered through the cerebral cortex.

In the cortical cells the changes are largely nuclear, though occasionally shrunken, acidophilic necrotic cells are found. In the basal ganglia necrosis of cells is conspicuously in evidence. The necrotic cells occur in irregular groups.

The common and conspicuous nuclear change consists in the presence of masses of amorphous, finely granular, acidophilic material within the nucleus, associated with granules of basophilic material irregular in size. Sometimes, though less frequently, the acidophilic mass is single, occupies the center of the nucleus and is separated from the nuclear membrane by a clear zone.

More frequently, however, the acidophilic material is found in several masses, either almost filling the nucleus, or separated from the nuclear membrane by a clear zone. It is quite characteristic of these acidophilic inclusions that they incorporate amorphous granules of basophilic material. At times there is a large granule of basophilically-stained material which suggests a nucleolus. The central eosinophilic mass is not usually separated so clearly from the nuclear membrane by a clear zone, neither is the aggregation of chromatic particles upon the nuclear membrane so characteristic as in herpes. Quite commonly the rarefied zone about the inclusions is not distinctly clear. The acidophilic material seems finely granular in composition and only loosely adherent.

Occasionally one finds the entire nuclear content apparently coagulated into a coarsely granular clump in the center, separated from the nuclear membrane by a clear zone. These nuclear clumps stain basophilically and do not seem to be identical with the commoner acidophilic inclusion. I have observed similar clumping of basophilic nuclear material in sections of brains from apparently normal fowls. Only ganglion cells contain inclusions. No change is observed in neuroglia, ependyma, choroid plexus or endothelium.

Blood Vessels: These are generally congested, and punctate hemorrhages are found both in the cortex and basal ganglia, but more frequently in the latter.

Exudate: There is no diffuse cellular exudate. About some of the small distended veins of the basal ganglia there are collected a few mononuclear cells difficult to classify, and there is an occasional small group of mononuclear cells in a focus related to necrotic ganglion cells. In a superficial inspection of the section one would hardly detect any cellular exudate at all, notwithstanding the extensive

neuronic degeneration and necrosis. There is no exudate in the meninges. The cellular degeneration is diffuse, extensive and bilateral.

Cerebellum and Pons: Similar cellular changes are abundant in the pons. No definite changes are seen in the cerebellum. Purkinje cells contain much granular eosinophilic coagulum in the nucleus, but this appears to be normal.

Adult Mouse Brain

Section Through Cerebral Cortex, Basal Ganglia and Ammon's Horn: One can easily recognize under low magnification that there is a diffuse encephalitis throughout the midcerebrum. This is indicated by an abundant perivascular cellular infiltration, very marked in the basal ganglia, and inconspicuous in the cortex. There is moderate round-cell infiltration in the meninges at the base of the brain. The inflammatory lesions are bilateral in distribution.

Blood Vessels: The blood vessels, including capillaries, are congested and occasional punctate hemorrhages are seen in the basal ganglia.

There is an abundant perivascular cellular infiltration about the larger veins. The cells are all mononuclears, some of them are lymphocytes, but most are wandering cells. No polymorphonuclear leucocytes are seen. In addition to the perivascular exudate there is also a diffuse invasion of parenchyma by mononuclear wandering cells. This is most abundant about the mantled veins and capillaries. An occasional polymorphonuclear leucocyte is seen in the parenchymal exudate. Now and then a mitotic figure is found in a neuroglial cell.

Cellular Changes: Despite the abundant perivascular and diffuse cellular exudation, neuronic alterations are difficult to find. There are occasional necrotic cells, but very careful search is necessary to discover a nuclear inclusion. They are almost negligible in number in the cerebral cortex and midbrain where perivascular infiltration is most marked, but are fairly numerous and typical in Ammon's horn, where there is no inflammatory exudation.

Encephalitis of Monkeys

No distinct differences could be noted between the severity, extent or general characteristics of the lesions in the brains of monkeys, whether they received directly the virus from infected mouse brains, or serial inoculations of brain from other monkeys infected with the mouse virus. Each of the brains of Groups II and III shows an extensive, severe, bilateral acute encephalitis which affects especially the gray matter, and in general seems most intense in the basal ganglia and pons. Altogether there were nine monkeys in these two groups. In addition to sections of the brain there was also tissue from the spinal cord from five of the nine monkeys. In four of these there is a severe, destructive, acute myelitis affecting the entire gray matter of the cord, but particularly involving the motor ganglion cells of the anterior horns. In one spinal cord no inflammatory changes were observed. The sections, however, were taken from one level only.

An illustrative protocol of the encephalomyelitis in monkeys follows:

Rh-282. Mouse Brain to Monkey Brain

Cerebral Cortex: In the plane of inoculation there is an extensive degeneration and necrosis of ganglion cells. The nuclei of many of these cells contain rather coarse acidophilic clumps or inclusions. It is difficult or impossible to recognize in these inclusions anything characteristic of yellow fever. Certainly one would hesitate to make a tentative diagnosis of yellow fever encephalitis on that basis.

There is a diffuse, though moderate polynuclear leucocytic infiltration of the cortical tissue, and to a less extent a mononuclear wandering cell exudate. About many blood vessels there is a thin perivascular mantle of mononuclear cells. The vessels generally are greatly dilated and distended. Petechial hemorrhages are numerous. Edema is evident. There is no meningitis.

Cerebellum and Pons: The cells of the cerebellum show no recognizable changes. The pyramidal cells of the normal monkey's cerebellum contain relatively conspicuous eosin-staining clumps and granules about the nucleolus. There is no inflammatory exudate in the cerebellar cortex.

The gray matter of the pons beneath the cerebellum shows, particularly within and about groups of large ganglion cells, an ex-

tensive degeneration, necrosis and inflammatory exudate. In the nuclei of relatively intact ganglion cells showing chromatolysis occasional distinct acidophilic inclusions were observed. These sometimes correspond in appearance to the yellow fever inclusion in general. Other nuclei, perhaps more commonly, contain in addition to the nucleolus one or more compact, spherical or oblong, pink-staining, homogeneous masses somewhat larger than nucleoli, situated in the center of the nucleus and separated from the nuclear membrane by a clear zone. Upon the nuclear membrane lie particles of basophilic material. These inclusions are not typical of yellow fever.

Many ganglion cells are shrunken and necrotic, and apparently they may reach the stage of necrosis without exhibiting the change characterized by inclusions. Early in the degenerative stage mononuclear phagocytic cells appear about the periphery of the cell, and soon entirely replace it. Occasionally a mitotic figure is seen which seems to be in a neuroglial cell. Of especial interest is the observation that not infrequently an acidophilic inclusion is to be found within the nucleus of one or more of the mononuclear inflammatory cells which are phagocyting a ganglion cell. These structures are round or elongated, and are about the size of a nucleolus, but distinct from it. They appear more dense, concrete, and refractive than the acidophilic inclusions generally seen in the ganglion cells, but are no more so than others that are occasionally found.

Polymorphonuclear leucocytes are rarely found in this section. There is a diffuse infiltration of the affected gray matter by mononuclear wandering cells with pale irregular nuclei, and a slight perivascular accumulation of similar mononuclears and lymphocytes. Veins are conspicuously distended.

Right Hemisphere and Brain Stem: One-half the brain, including the pons and medulla, was available in formalin, to study the distribution of lesions on the side opposite that receiving the inoculation.

Transverse sections including the entire half brain were cut through the frontal, parietal and occipital lobes, and through the cerebellopontine portion and through Ammon's horn. A diffuse encephalitis with lesions similar to those described, though varying in extent, was found in the gray matter from the frontal region through the pons. In some areas, such as Ammon's horn, the inflammatory exudate consists almost entirely of polymorphonuclear leucocytes.

Spinal Cord: There is ganglionic necrosis in both ventral horns. Several of these cells are being phagocyted by mononuclear phagocytes. There is a diffuse inflammatory exudate consisting of both polynuclear and mononuclear leucocytes. Edema and petechial hemorrhages are found.

Comment: An examination of these nine monkey brains shows that the strain of virus derived by inoculating mice intracerebrally with yellow fever virus and passed serially through these animals is an exceedingly destructive infectious agent for the central nervous system of normal monkeys, whether the virus is introduced directly from the mouse or passed serially through the brains of monkeys. One is led to judge that the virus rapidly traverses the central nervous system from the site of inoculation, causing an intense encephalomyelitis. The meninges do not seem to be involved in the inflammatory process.

The infectious agent attacks primarily, if not exclusively, the neurons (both sensory and motor), resulting in rapid degeneration and necrosis of these cells before inflammatory exudate appears. Associated with the injury to ganglion cells there is congestion of capillaries and veins, an inflammatory edema, and focal hemorrhages.

In what seem to be unusually severe acute lesions polymorphonuclear leucocytes make their appearance early and in considerable numbers before mononuclear phagocytes are to be found. Not only may there be a diffuse distribution of polynuclears in the inflamed area, but not infrequently they localize about dead ganglion cells. More commonly, however, there is an admixture of large mononuclears or the cellular exudate is composed of them entirely. Ordinarily phagocytosis of dead ganglion cells is accomplished by the mononuclears entirely. It is in these inflammatory cells collected about or replacing dead ganglion cells that one occasionally sees an acidophilic intranuclear inclusion, the significance of which is not apparent. If the lesion is of sufficient duration the veins become mantled by an accumulation of mononuclear cells, for the most part lymphocytes.

It seems evident in these preparations that ganglionic injury and necrosis is the first manifestation of the destructive effect of the virus in the nervous system, and inflammatory reaction, including cellular infiltration, is secondary.

In comparison with other neurocytotropic virus lesions, the mouse virus encephalitis resembles that of herpes in the rabbit and polio-

myelitis in man and monkey, rather than that of rabies and Borna disease. Like herpes and poliomyelitis the mouse virus causes a very acute fulminating disease, acutely destructive of ganglion cells. Unlike poliomyelitis, however, the injury is not so restricted in its distribution and affects both sensory and motor cells. In its distribution and its action upon both sensory and motor neurons it is more like the herpetic encephalitis, as seen in fulminating infections of rabbits. Unlike herpes, however, the mouse virus does not seem to affect the meninges, and its lesions are not so focal.

Nuclear Inclusions in Mouse Virus Encephalitis of Monkeys

Of especial interest in this study is the cytology of the neurons affected by the virus, with particular reference to the occurrence of intranuclear inclusions in the brains of monkeys. The observation by Theiler that intranuclear inclusions similar to those described in yellow fever occur in the brains of infected mice, and the presence of such inclusions in the mouse brains sent to me by Dr. Sellards, led to the expectancy that the mouse virus encephalitis of monkeys would be easily distinguishable from other forms of acute encephalitis by the presence of specific inclusions in ganglion cells. This, however, did not prove to be the case. It is true that intranuclear inclusions have been found in my preparations, but they are usually detected with difficulty, are few in number, and are often distinctly different morphologically from the typical intranuclear inclusions of yellow fever livers and mouse brains infected with the yellow fever virus. All of the inclusions observed were intranuclear and they were found in only five of nine cases of encephalitis in monkeys. My search was not exhaustive, however, and an investigation of more slides from different blocks of tissue possibly would have revealed a higher incidence. The inclusions found impress one as being of viral origin and occasionally they appear typical of yellow fever. The variations from type were found particularly in large multipolar ganglion cells, in one case the motor ganglion cells of the anterior horns of the spinal cord. In such cells there is chromatolysis and an eosinophilic staining of the cytoplasm. The nucleus is perhaps slightly enlarged. The nucleolus is partially or completely preserved, staining with methylene blue. About the nuclear mem-

brane are aggregated amorphous basophilic particles. The remainder of the nucleus appears empty except for one or more spherical or oblong, homogeneous, hyaline, pink-staining masses, usually larger than the nucleolus. Sometimes there are in addition to these structures smaller aggregations of minute pink particles resembling the material which constitutes the typical yellow fever inclusions. This description is based upon sections fixed in Zenker's solution and stained with methylene blue and eosin. In smaller ganglion cells the central area of the nucleus is sometimes found to be filled with pink-staining granular material, with chromatin particles collected upon the nuclear membrane. In these cells no nucleolus could be detected. Usually necrotic cells show no evidence of inclusions.

In this investigation only the staining reaction of inclusions with eosin, and their morphology, have been considered, partially because of the limited possibilities of the material at my disposal. In an exhaustive and detailed investigation of the yellow fever inclusions of human and monkey livers Cowdry and Kitchen were unable to detect any microchemical differential characteristics of these structures, and they finally relied largely, as did Torres, upon morphological configuration for evidences of specificity.

Discussion

The discovery by Theiler that the encephalitic brains of mice inoculated with the virus of yellow fever contain intranuclear bodies similar in every way to the inclusions previously described by Torres, and by Cowdry and Kitchen in the livers of monkeys and human beings dead of this disease, is very strong evidence that this cellular change is a characteristic effect of yellow fever virus upon cells. Especially significant is the fact that similar changes are brought about in two tissues so different as those of the liver and the brain. This cytological characteristic of the lesion, together with the immunological data supplied by the experiments of Theiler and of Sellards, makes it seem very probable that the virus of mouse encephalitis induced by the inoculation of typical yellow fever virus is in reality a modified form of the active agent of yellow fever.

It seems to be a unique phenomenon that a virus can become so distinctly and rigidly changed in its tropism or cellular relationship, although experience affords several instances of the variability of

viruses induced by experimental procedures, such as the modification of smallpox virus by passage through the calf. Such changes, however, represent apparently only variations in virulence, not of cytotropism. The virus of herpes simplex affords an instance of a profound divergence in cytotropic affinity of a virus in different species, as manifested by strains which possess a predilection for the skin in human beings and nervous tissue in the rabbit. The herpes virus, passed serially through the brains of rabbits, according to the experiments of Teissier, Gastinel and Reilly,[8] tends to lose its infectiousness for the human skin, but there is no indication that herpes virus becomes thereby more neurotropic in the human.

There is no reasonable doubt that Theiler's mouse virus is a neurocytotropic virus. It corresponds in its pathological activity and tissue affinity to the viruses of rabies, poliomyelitis, Borna disease and herpes. It is rather unexpected therefore that the mouse virus inoculated into the brains of the monkey (*M. rhesus*) does not induce more characteristically the cellular changes found in the brains of mice infected with the virus of yellow fever. However, there is a variation in the observed incidence of the yellow fever inclusions in the livers of human beings and of monkeys. The most distinctive difference between the effect of the mouse virus in the brains of mice and in the brains of monkeys is in the morphology of the intranuclear inclusions. Although one occasionally finds inclusions in encephalitic monkey brains which may be interpreted to be similar to those of mouse encephalitis, it is more common to find intranuclear inclusions, apparently of viral origin, which differ from them. An intranuclear inclusion in the monkey encephalitis atypical of yellow fever is a more compact, homogeneous, and discrete acidophilic mass somewhat suggestive of that found in Borna disease, though usually larger.

It should be borne in mind, however, that there is considerable morphological variation in most inclusions, though similar variations occur in each tissue regardless of the species of the host. The yellow fever inclusions are especially difficult to diagnose with certainty in their finely granular form as observed in fixed tissue, because they resemble so closely the granular precipitate from nucleoplasm which may be observed in many normal nuclei. They may be recognized with certainty, as is true also with the herpetic inclusions, only in their well developed forms, and when they occur in numbers.

The difference in structure of the typical intranuclear inclusions of the encephalitis of monkeys inoculated with mouse virus may represent, therefore, another variation in the activity of this virus not commonly seen in viral diseases.

In consideration of the protection experiments of Theiler and of Sellards, and of the fact that intranuclear inclusions quite like those of yellow fever are demonstrable in the cells of mouse brains inoculated with yellow fever virus, and finally that a fatal viral encephalitis may be induced in monkeys by the intracerebral injection of virulent mouse brains, characterized by the presence of intranuclear inclusions (some of which may resemble those of yellow fever) one feels that the evidence, both immunological and cytological, favors the view that the mouse virus represents a modified strain of yellow fever virus.

It is felt, however, that monkey encephalitis induced by mouse virus should be much more carefully studied from the viewpoints of its cytology, and of the cellular relationship and distribution of the virus.

Summary

1. A histological and cytological study has been made of an encephalitis of monkeys (*M. rhesus*) inoculated intracerebrally with the mouse strain of yellow fever virus.

2. The lesion in the monkey's brain is an acute, disseminated encephalomyelitis, extending apparently throughout the central nervous system, affecting the cellular tissues and causing necrosis of ganglion cells, both sensory and motor.

3. Intranuclear inclusions sometimes resembling, but more often differing from, those characteristic of yellow fever have been demonstrated in ganglion cells of the encephalitic monkey's brain.

4. On immunological and histological grounds it is judged that the virus of mouse and monkey encephalitis represents a biologically modified strain of yellow fever virus.

5. Cytologically the evidence of morphologically characteristic yellow fever intranuclear inclusions in the brains of encephalitic monkeys inoculated with the mouse virus is inconclusive.

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DESCRIPTION OF PLATES

Magnification 2300, except Fig. 3, which is 60 diameters.

Plate 24

Fig. 1. Ganglion cells from baby mouse's brain. Intranuclear granular acidophilic inclusions interspersed with basophilic granules. The larger ones may be nucleoli. These inclusions are like those of yellow fever livers.

Fig. 2. Ganglion cell from baby mouse's brain. Intranuclear mass largely composed of basophilic granular material. Atypical of yellow fever.

Fig. 3. Monkey encephalitis, to show perivascular and diffuse cellular infiltration.

Fig. 4. Ganglion cell from monkey encephalitis showing nucleolus (dark sphere) and acidophilic granular material rather loosely arranged, resembling yellow fever inclusions.

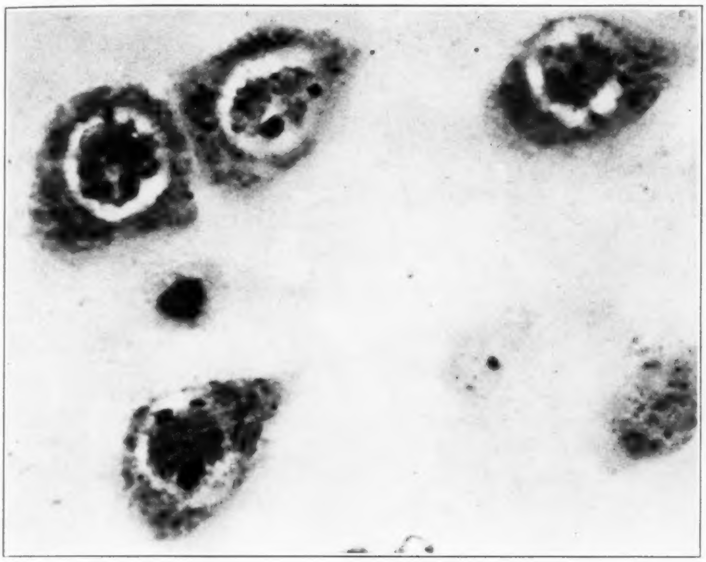

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PLATE 25

FIG. 5. Monkey encephalitis to show phagocytosis of dead ganglion cell by polynuclear leucocytes.

FIG. 6. Same showing diffuse polynuclear exudate.

FIG. 7. Ganglion cell from monkey encephalitis showing oblong compact acidophilic intranuclear inclusions, at one end of which is a nucleolus. Note clear intranuclear space and aggregation about nuclear membrane of basophilic particles. Atypical of yellow fever.

FIG. 8. Ganglion cell from monkey encephalitis showing nucleolus and acidophilic masses or inclusions, atypical of yellow fever.

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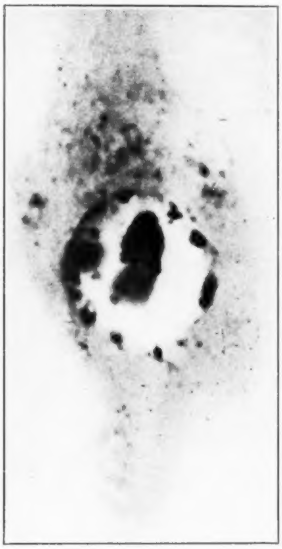

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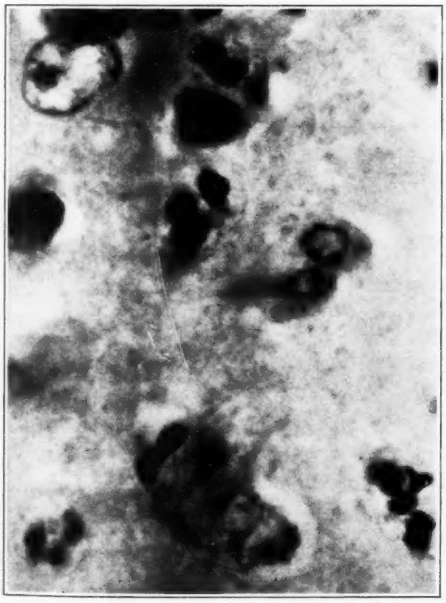

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Goodpasture

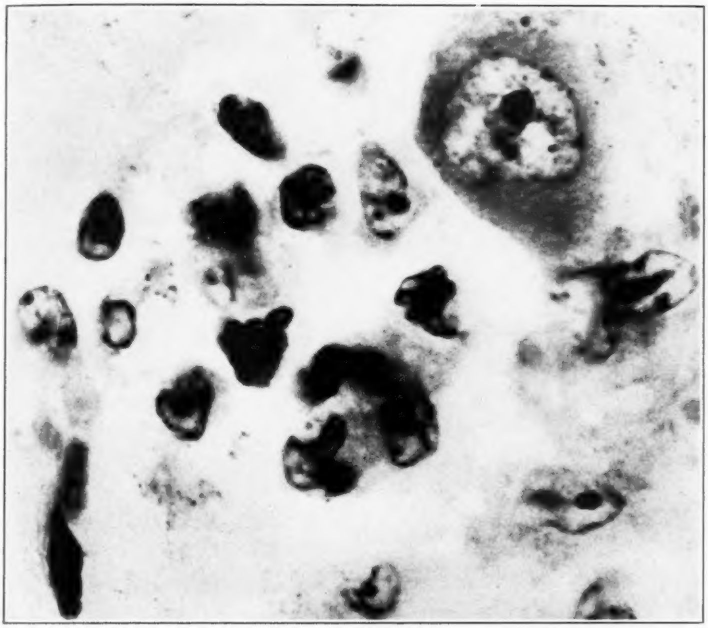
Yellow Fever Encephalitis of Monkey

PLATE 26

FIG. 9. Monkey encephalitis. Phagocytosis of dead ganglion cell by mononuclear leucocytes.

FIG. 10. Necrotic ganglion cells of monkey encephalitis. Central eosinophilic material filling nuclear space. Basophilic particles upon nuclear membrane. Atypical of yellow fever.

FIG. 11. Large ganglion cell from monkey encephalitis showing large eosinophilic masses. The basophilic nucleolus is incorporated in the mass to the left. Atypical of yellow fever.



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THE OCCURRENCE OF INTRANUCLEAR INCLUSIONS IN MONKEYS UNACCOMPANIED BY SPECIFIC SIGNS OF DISEASE *

W. P. Covell, Ph.D.

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Stewart and Rhoads [1] in 1929 reported the discovery of intranuclear inclusions in the cells of the nasal mucous membrane of monkeys (*Macacus rhesus*) inoculated with the virus of poliomyelitis, but since they also found them occasionally in normal control monkeys they did not attach any particular significance to them. While making a detailed study of the lesions in experimental poliomyelitis I have encountered similar inclusions, not only in the situation mentioned by Stewart and Rhoads but, in addition, in the epithelial cells of the trachea, lungs and bile ducts. These intranuclear inclusions were likewise seen in animals which had not been subjected to the virus of poliomyelitis. I venture to report my findings briefly, because it is desirable to be as fully informed as possible concerning the presence of such inclusions unaccompanied by clinical symptoms in an animal like the monkey, which is used so much in the study of filterable viruses. The material on which this report is based was secured from 60 monkeys as follows:

Experimentally infected with poliomyelitis	37
Experimentally infected with nasal washings from cases of measles	5
Diarrhea	9
Generalized tuberculosis	2
Pneumonia	3
Normal	4

Autopsy was performed promptly after the animals died from disease or were killed with ether. Small pieces of the nasal mucous membrane, trachea, lungs, liver and other organs were fixed for twenty-four hours in Zenker's or Helly's fluid, and, after the usual

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procedures, were stained with erythrosin-azur or phloxine-methylene blue.

The occurrence of intranuclear inclusions is indicated in the table, in the sites mentioned, by plus (+) signs. No inclusions were observed in other parts of the body. The minus (−) signs refer to negative observations and the zero (o) signs to instances where no

TABLE I

The Occurrence and Distribution of Intranuclear Inclusions in the Respiratory Tracts and Bile Ducts of 20 Monkeys.

Monkey No.	Disease	Nasal mucous membrane	Trachea	Bron-chioles	Alveoli of lungs	Bile ducts of liver
5	Poliomyelitis	−	o	−	+	−
12	"	+	o	−	−	−
34	Normal	−	−	−	+	−
38	Poliomyelitis	−	+	+	−	+
77	"	o	o	−	+	−
90	Measles	−	−	+	+	+
115	Diarrhea	+	−	−	−	−
116	Measles	−	−	−	+	−
123	"	−	−	−	+	−
135	Diarrhea	o	o	−	+	−
143	Poliomyelitis	o	−	−	+	−
156	"	o	−	−	+	−
164	Tuberculosis	−	+	−	−	−
168	Poliomyelitis	−	o	−	+	+
180	"	+	+	−	−	−
187	"	+	−	−	−	−
188	Diarrhea	+	−	−	−	−
195	Poliomyelitis	+	−	−	−	−
199	"	o	−	−	+	−
208	Diarrhea	−	o	−	+	−

tissue was available for study. Intranuclear inclusions were found in 20 out of the 60 monkeys. It is possible that still more might have been seen had serial sections on a large scale been examined. The distribution of inclusions was patchy. In only 1 monkey (No. 90) were they detected in 3 of the 5 locations listed in the table. In 2 others they were seen in 2 sites, while in the remaining 17 they were discovered only in 1 position. The locations in order of frequency were: alveoli of lungs (12), nasal mucous membrane (6), trachea and bile ducts (3 each), and bronchioles (2). It may be significant that inclusions were found in the bile ducts of the livers in the mon-

keys that also exhibited them somewhere in the respiratory tracts. However, seventeen monkeys, in which inclusions were noted in the respiratory tract, did not reveal them in the bile ducts. The number of positive observations is far too small on which to base any conclusions as to the primary site of action of the virus and its subsequent spread, if, as seems probable, a substance of this kind is involved. Neither does the number of animals examined permit any valid correlation between the type of disease and the incidence of inclusions as a possible accessory factor in rendering the tissue susceptible to the action of a hypothetical filterable virus. But it is to be noted that they were seen in 60 per cent of the animals experimentally infected with the measles virus, and in none of the animals which suffered from pneumonia.

The properties of the inclusions in the 5 locations were much the same. As seen in the nasal mucous membrane they corresponded in every particular with those previously reported by Stewart and Rhoads. They were most numerous in the more superficial epithelial cells, the nuclei of which were affected in varying degrees. In some, only a slight increase in nuclear acidophilic material was noticed, which of itself could not be regarded as noteworthy, but in many cells the process was carried to an extreme with: (1) characteristic margination of all basophilic material on the inner surface of the nuclear membrane, (2) clumping of the acidophilic material in the center of the nucleus, and (3) the appearance of a clear halo between the inclusion and the nuclear membrane. Such intranuclear inclusions were not seen scattered evenly throughout the extent of the mucous membrane, but were definitely restricted to a few small areas characterized by a necrosis and sloughing of the membrane, which in two cases was accompanied by an inflammatory reaction in the underlying tissues, including the glandular epithelium.

In the trachea, also, the inclusions were limited to similarly injured areas. An intranuclear inclusion in this situation is illustrated in Fig. 1. It is contained in a rather large nucleus slightly to the left of the center. At first sight it might be taken for an enlarged nucleolus, but careful examination showed that it was formed through the clumping together and fusion of acidophilic particles. Another nucleus just to the left is in an earlier stage of the reaction, being filled with finely divided acidophilic material. Photomicrographs like this, taken at a magnification of only 1500 diameters, give obvi-

ously a very incomplete impression of the details, for the color contrast which is so distinctive in original preparations is suppressed and the information to be secured by focusing is lacking.

Fig. 2 illustrates a multinucleated cell bordering the lumen of a bronchiole in each nucleus of which a typical inclusion is to be seen. One is thereby reminded of the appearance of intranuclear inclusions in chickenpox. The bronchioles which possess inclusions are often infiltrated with mononuclear leucocytes and in one instance a mite was found in close proximity, the tissue about it being inflamed.

The intranuclear inclusions discovered in the epithelial cells of the alveoli of the lungs, and in occasional cells free in the lumen, were of more striking appearance. One of them is shown at about the center of Fig. 3. The inclusion itself is roughly spherical, fairly compact and separated from the nuclear membrane by a marked halo. Such inclusions were more abundant in some lungs than in others. Though distributed in an irregular way, being more numerous in certain areas, they were not associated with detectable lesions, as in the other parts of the respiratory system.

Also in the intrahepatic bile ducts the inclusions were observed in the absence of distinctive tissue changes, other than repeated nuclear division (Fig. 4).

Discussion

That these inclusions in the respiratory tract and bile ducts are caused by some virus of low virulence is a fair assumption, because despite repeated experiments by many investigators nuclear alterations of this kind have never been produced by agents other than viruses. Cole and Kuttner [2] go so far as to state in their paper on intranuclear inclusions in the salivary glands of guinea pigs: "It is true that Luger and Lauda have mentioned the occurrence of similar structures in a case of salvarsan dermatitis. Even though these lesions should be present in isolated instances of this kind, it would be necessary to demonstrate the absence of a filterable virus in the given instance before the present conception of the direct relationship between these nuclear changes and filterable viruses would become untenable." These investigators hold that the presence of a filterable virus is to be assumed when typical intranuclear inclusions occur, unless it is possible to prove the absence of a virus experimentally.

If, then, we accept the inclusions as caused by virus action, the next question is whether we have to do with one virus spreading along the respiratory tract to all of the locations described, or with two viruses. This extension might be easily understood in the case of the respiratory tract, but how the bile ducts without corresponding alterations in intervening parts of the body come to be involved if only one virus is operating, is difficult to explain. I merely mention the possibilities without hazarding any interpretation. The indications are stronger for the operation of two viruses in man because the inclusions in visceral disease (VonGlahn and Pappenheimer [3]) are very different from those seen in the salivary glands (Goodpasture and Talbot [4]). Even in these cases we are not justified in reaching a definite conclusion as to the presence or absence of two viruses, because the difference in the resulting inclusions may be attributable to variations in the response of different types of cells to one and the same virus. The reason for making this qualification is that the submaxillary gland virus is known to produce enormous intranuclear inclusions with great nuclear hypertrophy in its usual site of action, namely, the ducts of the salivary glands of guinea pigs; and small inclusions with but slight increase in size, more closely resembling herpetic inclusions on intracerebral inoculation in guinea pigs.[2]

The nature of the virus or viruses, which leads to the development of the inclusions in the monkey described in this paper remains wholly unknown. The inclusions themselves are not for a moment to be compared with the other intranuclear inclusions in injured nerve cells in experimental poliomyelitis in monkeys, Covell,[5] and the existence of which has been confirmed by Hurst.[6] The latter are discretely rounded masses, which bear a resemblance to the inclusions pathognomonic of Borna disease.

The herpetic intranuclear inclusions produced in the liver cells of another species of monkey (*Cebus hypoleucus*) by Cowdry and Kitchen [7] are much less dense in consistency and may fill the entire nucleus. The intranuclear inclusions caused by the virus of yellow fever in rhesus monkeys, likewise in liver cells (as noted by the same authors) are also different, in that they are laid down in clusters of discrete particles which do not typically fuse into a single mass. The difference may be due perhaps to the fact that different kinds of cells are responding, for Magalhães [8] found intranuclear inclusions in the renal cells of monkeys experimentally infected with the virus of

yellow fever, which more closely resemble those that I have seen. At present no other intranuclear inclusions are known in monkeys, with which to make a comparison.

Conclusions

From these observations and those of Stewart and Rhoads, it seems likely that monkeys must now be listed with humans,[3] guinea pigs,[4] rats (Thompson [9]), rabbits (Rivers and Tillet [10]), and dogs (Cowdry and Scott [11]) as animals in which one or more viruses sometime lurk, capable of producing intranuclear inclusions in the absence of recognizable clinical symptoms. Particularly is this of interest when monkeys are employed for experiments with viruses, which in the proper environments are definitely disease-provoking like those of poliomyelitis, yellow fever, chickenpox and measles.

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DESCRIPTION OF PLATE

Plate 27

Photomicrographs of intranuclear inclusions in the respiratory tract and bile ducts of monkeys taken at a magnification of 1500 diameters.

Fig. 1. Type of intranuclear inclusion in the trachea. The cytoplasm of the cell is deeply stained. A compact, rounded, acidophilic mass is located centrally in the nucleus.

Fig. 2. Intranuclear inclusions in the epithelium of a bronchiole. The nuclei have increased in number to form a giant cell with a tendency to margination of the basophilic chromatin. The inclusions are less compact than those in the alveolar epithelium and are in the form of discrete particles separated from the remaining chromatin by clear areas.

Fig. 3. Intranuclear inclusions in the lung. Centrally located in the field is a nucleus of an alveolar epithelial cell containing an inclusion body. The monkey from which this photograph was made had received an intracerebral inoculation of poliomyelitis virus and was sacrificed during the early paralytic stage of the disease. The intranuclear inclusion is separated from the basophilic chromatin by a clear area.

Fig. 4. Type of intranuclear inclusion found in the bile ducts of the liver. The basophilic chromatin is plastered against the nuclear membrane from which the inclusion is separated by a clear area. The similarity in the resemblance of this type of inclusion to that in the epithelium of the bronchioles is striking. The inclusions are in the form of clusters of discrete particles.

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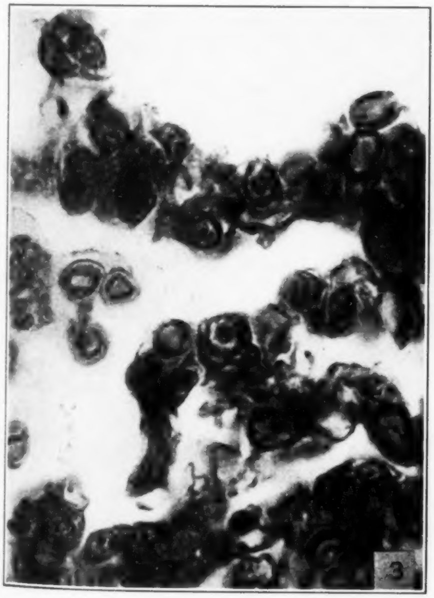

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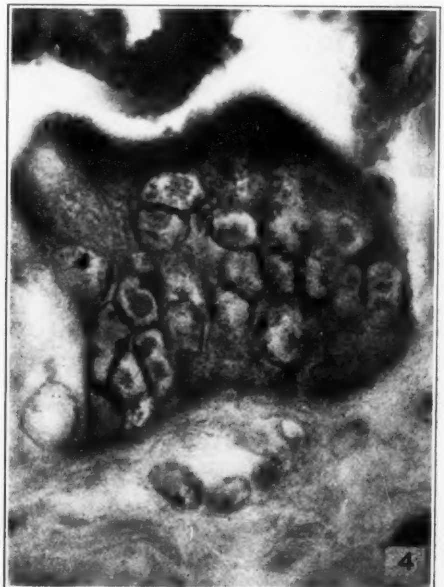

4

ANATOMICAL CHANGES IN THE LIVERS OF DOGS FOLLOWING MECHANICAL CONSTRICTION OF THE HEPATIC VEINS *

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This paper is a report of the changes observed in the livers of seventeen dogs whose hepatic veins were mechanically constricted for periods of 7 to 30 minutes for the purpose of studying the chemistry in the blood during the succeeding 24 to 72 hours. Practically all of the recorded anatomical studies of the liver following alterations in the hepatic circulation have been based upon permanent changes in the blood flow and, therefore, are concerned with more or less chronic modifications of that organ. Thus, the results of ligation of the hepatic artery have been investigated by Holst,[1] Behrend, Radasch and Kershner,[2] Ritter,[3] Hori,[4] Loeffler,[5] and others. Bainbridge and Leathes,[6] de Josselin de Jong,[7] Rous and Larimore,[8] Papilian,[9] Chiari,[10] and others, have studied the effect upon the liver of either ligation or thrombosis of the portal vein. Zimmerman and Hillsman [11] placed metal rings about the vena cava between the entrance of the hepatic veins and the heart. Hess,[12] in 1905, and more recently Satke [13] and Saborowsky [14] have reviewed the literature and discussed the results of obliterating endophlebitis of the hepatic veins. There are also occasional reports in the literature of alleged retrograde embolism of the hepatic veins (Heller,[15] Risel,[16] Meixner,[17] and Reiniger [18]). But in all of the above experiments and observations the alteration in the hepatic circulation was continuous. We have been unable to find any studies of the changes in the liver resulting from a sudden and complete, but temporary, closure of the hepatic veins.

Mechanical constriction of the hepatic veins by the method described by Simonds and Brandes [19] causes an immediate increase in the size of the liver, which becomes enormously distended with blood and dark brownish purple in color. This condition continues until

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the obstruction is released, when the liver promptly becomes smaller and somewhat paler. This procedure deprives the liver cells of oxygen, subjects them to a considerable increase in pressure, interferes with their nutrition and permits the accumulation of metabolic products in the surrounding medium during the period of constriction.

Of the seventeen dogs used in these experiments, one died 4 hours after the operation in typical hypoglycemic convulsions, two were sacrificed after 24 hours, eleven after 48 hours, two after 72 hours, and one after 7 days.

The liver weight-body weight ratio in these animals was distinctly increased, the mean being 0.0376 ± 0.0035, the individual ratios ranging from 0.025 to 0.047, only two being normal or below. Junkersdorf [20] found the liver weight-body weight ratio in normal dogs to be 0.030; Simonds and Brandes [21] obtained a mean ratio of 0.0303 in thirty-one normal dogs. The mean ratio in these animals was, therefore, approximately 25 per cent higher than the normal.

The increase in the weight of the liver was due in part to edema. Simonds and Brandes [22] observed an average increase of 2.5 times the normal outflow from the thoracic duct during mechanical constriction of the hepatic veins. On the basis of the microscopic examination of livers immediately, and 24 hours after constriction, it is assumed that much of this excess flow of lymph comes from the liver. The most marked change is in the lymphatics which surround the sublobular veins, many of which are encircled by widely dilated lymphatics filled with hyaline coagulated material (Fig. 1). These are apparently the radicles of the lymph vessels which follow the hepatic veins to the inferior vena cava, and thence along this vessel through the diaphragm into the posterior mediastinum. The connective tissue about these veins was rendered loose-meshed by accumulation of fluid between the cells. This can probably be accounted for as a result of damage to, and subsequent thrombosis of, the larger lymph vessels about the main branches of the hepatic veins. As shown by Opie [23] trauma is often an etiological factor in lymphatic thrombosis. The periportal connective tissue was also edematous.

Another element, of less importance, in the increase in weight of the livers of these dogs is the irregularly distributed increase of

blood. During constriction the amount of blood within the liver is enormous, but upon releasing the constriction most of the accumulated blood promptly escapes and when examined after 24 to 72 hours the liver as a whole is relatively poor in blood, with only a few scattered areas in which the central veins and adjacent sinusoids are distended with red cells.

On microscopic examination with low power one of the most striking features is the relative paleness of the central portion of the lobules (Fig. 2). The hepatic cells are swollen, more or less granular, many contain round clear spaces or vacuoles and some are without nuclei (Fig. 3). The vacuoles do not stain with osmic acid. The visible nuclei in this portion of the lobule vary greatly; some are swollen, extremely pale and washed out; others are compact and pyknotic, and relatively few are normal. The markedly swollen condition of these cells narrows, and, in places, practically obliterates the sinusoids so that the central part of the lobules is almost bloodless. At the periphery of the lobules is a zone of varying width in which the liver cells are more nearly normal. From this it appears that the hepatic cells in the central one-half or two-thirds of the lobule are less resistant to injury than those in the peripheral portion. A similar differential distribution of cell damage has been observed in other conditions, *e.g.*, chloroform poisoning, chronic passive hyperemia, and so on. In these conditions either a toxic agent or a disturbance of the circulation in the liver acts over a more or less long period of time. It has been suggested that the greater damage to the centrally located cells in the liver lobule is a result of their greater distance from the fresher part of the blood supply, the peripheral cells having the first opportunity to secure oxygen and nutriment from the blood as it percolates through the lobule, while the central cells receive only blood which has been depleted of substances essential to their life. But in our experiments the entire circulation through the liver was stopped temporarily. Hence both central and peripheral hepatic cells were subjected to identical conditions. The differential distribution of evidences of cell damage described above in the livers of our animals indicates a greater actual susceptibility to injury on the part of the cells in the central part of the lobules as compared with those of the periphery. Mallory [24] has suggested that the greater vulnerability of the central cells of the liver lobules is due to their greater functional activity

and higher degree of specialization. The results of our experiments tend to confirm this view.

A variable number of central and sublobular veins are filled with clear structureless masses, some of which stain blue, others dark red (Fig. 4). As a rule, lobules whose central veins are thus occluded contain more blood than the adjacent lobules.

A characteristic finding in all of these animals is the presence of masses of cells within the sinusoids (Figs. 5 and 6). These cells are of three types: an occasional lymphocyte, a few polymorphonuclear leucocytes and a greater number of mononuclear cells with large round, oval or indented nuclei and moderately abundant cytoplasm. These latter cells appear to have originated from proliferation of sinusoidal endothelium. These cell masses are either small and compact and lie in an oval dilatation of the sinusoid, resembling those described by Simonds [25] and by Manwaring, French and Brill [26] in anaphylactic and peptone shock, or they are larger and more diffusely and loosely arranged in several adjacent sinusoids, but consist of the same cell types as the above. Within this second form of cell masses the cords of liver cells are disrupted and many of the included hepatic cells are swollen, stain with eosin and are without nuclei. These areas therefore have much in common with the focal necrosis described by Mallory [27] in typhoid fever.

Cell groups of the first type are most numerous in the dog that was allowed to live for 7 days and whose hepatic veins were constricted for 20 minutes. In many of these masses, especially in the 24 hour dogs, a red hyaline matrix is easily visible. These compact intrasinusoidal masses are probably of the same nature as those designated by Pearce [28] as conglutination thrombi. Their manner of formation is probably as follows. During the stagnation of the blood in the sinusoids, while the hepatic veins are constricted, a group of red cells becomes packed into a firm mass which cannot be broken up when the circulation is restored. These later fuse into a hyaline matrix in which is entangled an occasional lymphocyte and into which may wander a few polymorphonuclear leucocytes. The presence of this "foreign body" within the sinusoid stimulates the proliferation of the adjacent lining endothelium from which is derived the chief part of the cell content of the mass.

In the second type of cell mass there is no evidence of fusion of red cells. The presence of a group of necrotic hepatic cells may

serve to stimulate the proliferation of the sinusoidal endothelium. If this interpretation is correct, the process is the reverse of that described by Mallory [27] as the probable pathogenesis of focal necrosis in typhoid fever.

In all these animals the Kupffer cells contained an abundance of brown, granular pigment resembling hemosiderin.

Summary

The livers of dogs examined 24, 48 and 72 hours and 7 days after mechanical obstruction of the hepatic veins for periods of 7 to 30 minutes showed the following changes.

1. A mean increase of 25 per cent in the liver weight-body weight ratio, due to edema and to swelling of the hepatic cells.
2. Swelling, granulation, vacuolization and extensive necrosis of the hepatic cells in the central half or two-thirds of the liver lobules.
3. Marked dilatation of the perivascular lymphatics surrounding the sublobular veins.
4. The presence of hyaline thrombi in many central and sublobular veins.
5. Intrasinusoidal cell masses of two types: (1) small, compact, occluding masses probably originating in "conglutination thrombi" of red cells, and (2) larger, more diffuse and branching cell masses.
6. Hemosiderosis of Kupffer cells.

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DESCRIPTION OF PLATE

PLATE 28

FIG. 1. Distention of the perivascular lymphatics about a sublobular vein. × 100.

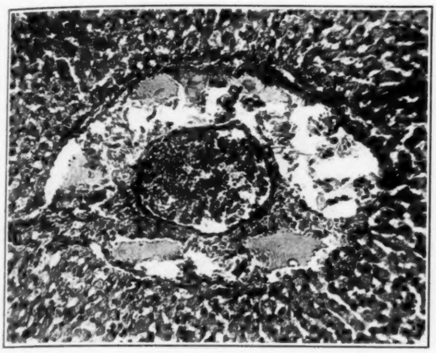
FIG. 2. Lower power field showing pale central portions of lobules. × 20.

FIG. 3. Swelling and necrosis of liver cells. × 325.

FIG. 4. Hyaline thrombus in central vein. × 200.

FIG. 5. Compact cell masses in sinusoids. × 160.

FIG. 6. More diffuse cell masses in sinusoids. × 200.



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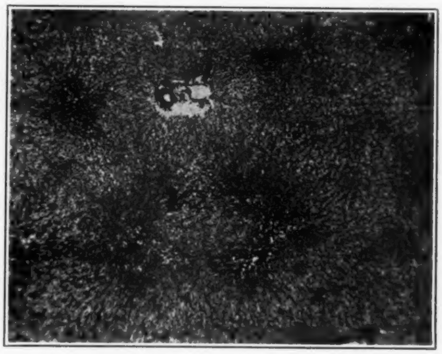

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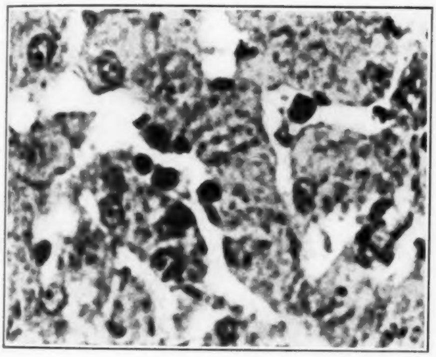

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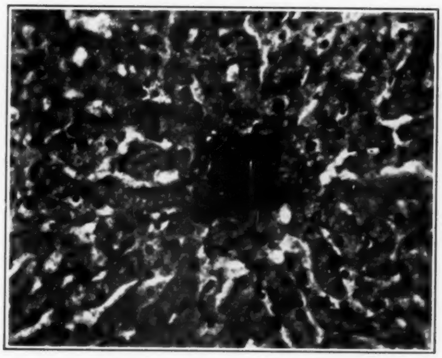

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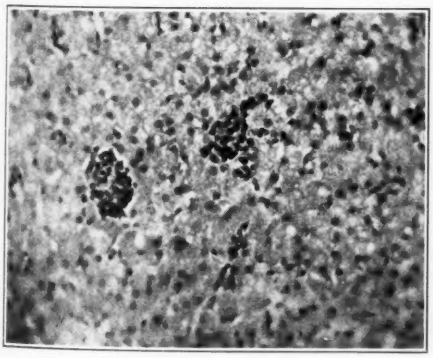

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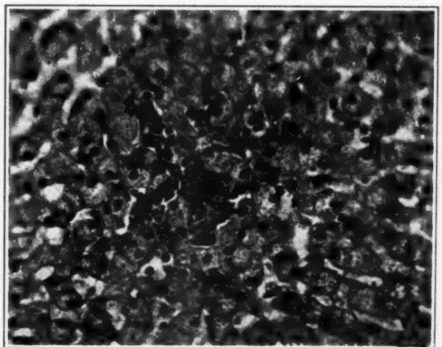

6

TORULA INFECTION *

A REVIEW AND REPORT OF TWO CASES

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Our knowledge of yeast infections in man originated in 1894 when Busse discovered a yeast-like organism in a leg tumor which had been diagnosed sarcoma of the tibia. At autopsy he found similar tumor masses in various tissues, from which he isolated the organism. Thinking he had discovered the etiology of neoplasms he studied various other types of tumors and found the organism in one sarcoma and several nasal polyps, but the yeasts proved to be nonpathogenic. Two years later Gilchrist called attention to a type of dermatitis produced by blastomycetes. Sanfelice isolated torulae from human malignant tumors and these strains in the hands of Nichols proved pathogenic for rabbits and guinea pigs. In 1902 Frothingham studied a tumor-like mass in the lung of a horse caused by blastomycetes, which he placed in the subdivision of torula. It was not until 1916 when Stoddard and Cutler reviewed yeast infections in man that a real attempt was made to correlate the clinical and pathological features of the disease with the morphological and cultural characteristics of the organism producing it. Following Wolbach they classified these infections as (1) true yeast infections, (2) coccidioidal granuloma, (3) oidiomycosis, and (4) torula infection. They selected from the literature four cases without skin or subcutaneous tissue lesions, characterized clinically by cerebral symptoms throughout the course of the disease. To these they added two cases of their own and classified them as examples of torula infection. In this group fever and leukocytosis are not constant. The meninges and often the brain, lungs, liver and spleen are affected. The organism varies from 1 to 13 microns in diameter and has a deeply staining wall, which in the larger forms appears as a double line. In tissues it buds but does not sporulate; in cultures it buds but never produces mycelia. The yeast produces a gelatinous

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material and stimulates a chronic inflammatory reaction in the meninges, which is apparently invariably fatal.

Since Stoddard and Cutler pointed out the necessity of bearing torula infection in mind in patients with increased intracranial pressure without localizing signs, chronic meningitis, or other obscure cerebral conditions, the number of recognized cases has steadily increased.

Report of Cases

Case 1. Mrs. F. M., 32 years of age, referred by Dr. Samuel Burrows, entered the Billings Hospital of the University of Chicago on Sept. 9, 1930, complaining of headache, pain and a feeling of tightness in the back of the neck, and pain behind the eyes. The family and marital history were unimportant.

Past History: In 1922, and again in 1928, the patient had pleurisy.

Present Illness: The patient's headache had been dull and inconstant for two years, but had become worse since the spring of 1929. A small lump in the right occipital region was noted in December 1929, from which a small cyst was removed in April 1930, and the bone scraped. The operation left a sinus which drained until June 1930, when at a second operation a small sequestrum was removed. On recovery from the ether anesthesia the headache, pain and feeling of tightness in the back of the neck developed and has persisted. Discomfort and a feeling of pressure behind the eyes has been noted for two months. During the two weeks before admission to the University Clinics nausea and vomiting occurred almost daily. The sinus in the occipital region has continued to drain.

Physical Examination: The patient was a rather thin, nervous woman, not acutely ill. In the right occipital region was a discharging sinus 2 mm. in diameter, which could be probed almost to the bone. Roentgen-ray examination showed no abnormalities of this area. The left maxillary antrum was cloudy and pus could be aspirated from it. Areas of increased density in both upper lung fields and marked enlargement of the mediastinal structures could be seen in roentgenograms, in spite of negative physical findings.

There was a slight stiffness of the neck but Kernig's sign was negative. Papilledema was 1/2 D on the right, and 1 D on the left with several exudative and hemorrhagic areas about the disk retinal margin on the left side. All of the other cranial nerves were normal. Sensation over the entire body was intact. All extremities were somewhat weak but the muscle tone was normal. The deep and superficial reflexes were normal, except for an absent right upper abdominal.

Course in Hospital: On admission to the hospital the temperature was 100° F, pulse 90, respirations 16, blood pressure 120/80; the white blood count was 9000, red blood count 5,100,000 and hemoglobin 90 per cent. The urine was normal and the Wassermann and Kahn tests on the blood were negative. On September 11, two hours after a rise in temperature to 101.6° F, the patient had a severe headache, vomited, talked irrationally and developed a generalized convulsion without focal signs; a second convulsion occurred thirty minutes later. The cerebrospinal fluid was clear and colorless with a pressure of 350 mm. of water and a cell count of 110 per cmm., of which 55 per cent were polymorphonuclears and 45 per cent lymphocytes. During the following five months the

TABLE I

Cerebrospinal Fluid in Case 1

Date	Pressure	Cell count	Poly-morpho-nuclear leucocytes	Lymphocytes	Total protein	Globulin	Sugar	Chlorides as NaCl	Lange	Culture	Wasser-mann
			per cent	*per cent*	*mg.*		*mg.*	*mg.*			
9/11/30	350	110	55	45	..	Trace	..	..	..	..	..
9/17/30	250	210	..	..	..	..	..	..	..	..	..
9/29/30	150	65	20	80	..	..	..	..	..	0	..
10/ 9/30	260	63	..	84 (16 l. m.)	85	Trace	Too low to read	758	..	0	..
10/20/30	Ventricle	14	30	70	54	..	20	691	..	..	..
10/23/30	400	24	16	84	..	Faint trace	5	674	..	..	..
11/10/30	320	43	27	73	108	Marked trace	15	674	1123211000	0	..
11/14/30	290	46	..	..	..	Trace	..	..	5555555421	0	Neg.
12/ 8/30	270	23	..	85 (15 degen.)	139	..	Too low to read	538	..	..	Neg.
12/30/30	Ventricle	20	50	30 (10 trans.) (10 l. m.)	..	..	..	..	..	0	Neg.
1/ 9/31	200	..	..	..	62	..	Too low to read	464	..	0	..
1/14/31	290	30	40	60	..	..	..	..	..	..	..
1/ 8/31	520	50	40	60	..	..	..	..	..	..	..

patient's progress was steadily downward. The temperature ranged from 100° to 103° F. There were numerous generalized convulsions, the papilledema increased to 5 D, nystagmus developed, and hypotonicity of the muscles of the extremities became marked. Drowsiness and mental confusion were outstanding. While in the hospital the occipital sinus was explored and the underlying bone and dura mater appeared normal. Later, ventriculograms disclosed a slight hydrocephalus but an otherwise normal ventricular system. In December Dr. Percival Bailey made a burr hole behind each mastoid process in the suboccipital region and probed the cerebellar hemispheres without finding pus. About two weeks before death, which occurred Jan. 29, 1930, retraction of the neck and a bilateral Kernig's sign were noted. The cerebrospinal fluid examinations are given in Table 1.

Autopsy Report

The autopsy was performed by Dr. Paul Cannon five hours after death. There were about 50 cc. of a bloody fluid in the left pleural cavity and about 100 cc. in the right. Fibrous adhesions were present between the parietal and visceral pleurae at both apices but were more marked in the left, these being extremely dense. Otherwise the pleural surfaces were smooth except for small firm nodules 2 mm. to 4 mm. in diameter, surrounded by fibrous tissue, scattered uniformly throughout both upper lobes. The lungs were less crepitant than normal, especially posteriorly. The cut surface of the left lung showed numerous fibroblastic nodules scattered especially through the upper third of the upper lobe. There seemed to be no fresh areas of tuberculosis of either lobe. The right lung showed a dense fibroblastic tuberculosis extending through the medial third of the upper lobe. There was some fibrocaseous tuberculosis in the lower lobe.

The tracheobronchial lymph nodes were fibrocaseous. Some of the inferior glands on the right were 3 cm. in diameter.

The spleen weighed 400 gm. It was soft and was adherent to the left side of the diaphragm by fibrous adhesions. When sectioned, the surface was somewhat paler than normal and many tiny white nodules were present. These nodules were uniformly distributed throughout the pulp and were about 1 mm. in diameter.

Together the adrenals weighed 14 gm. The left adrenal was slightly larger and paler than normal. The cut surface showed the cortex to be pale. The right adrenal resembled the left in size and appearance. An occasional small scar was seen on stripping the capsule of the kidney. Otherwise the kidneys appeared normal.

The mesenteric and retroperitoneal lymph nodes were normal in appearance. The lymph nodes at the hilum of the spleen were definitely enlarged and contained small, white, pin-head-sized bodies resembling tubercles. The lymph nodes surrounding the common duct were markedly enlarged and showed areas varying from 1 mm. to 8 mm. in diameter, which were whitish and opaque in appearance.

Brain and Meninges: The dura mater was under some increased tension. The cerebral hemispheres were of equal size, normal shape, and the convolutions were moderately flattened. The leptomeninges, which had a rather dry appearance, showed numerous small, white, tubercle-like nodules from 1 to 2 mm. in diameter scattered through them (Fig. 11); they were more numerous over the frontal lobes. The pia-arachnoid was thickened everywhere; this was most marked in the perichiasmal region where it was greatly thickened and had a gelatinous appearance. This exudate extended over the pons and medulla and over the superior part of the temporal poles. Around some of the larger vessels, particularly those of the Sylvian and Rolandic fissures, was a yellowish white exudate (Figs. 1 and 11).

Frontal sections of the cerebral hemispheres disclosed a slight degree of hydrocephalus but an otherwise normal ventricular system. The aqueduct of Sylvius was patent and the fourth ventricle normal. The prolongations of the pia mater between the gyri were thickened and that overlying the hippocampus, gyrus dentatus and insula was 2 to 3 mm. in thickness (Fig. 1). No cystic cavities were present in the cortex, the basal ganglia or elsewhere in the brain.

In sections passing just anterior to the temporal poles a small cavum septum pellucidum was seen. The cerebellum, pons and medulla were grossly normal, except for the exudate over their surface.

Microscopic Examination

Spleen: The spleen contains great numbers of tubercle-like nodules and shows marked hyaline degeneration which closely resembles amyloid but does not stain with Congo red. Giant cells are numerous, some having nuclei arranged around the periphery, others having the nuclei clumped in one part of the cytoplasm. No organisms can be found, but the giant cells occasionally show spaces resembling the residues of digested organisms; distinct outlines or shells appear clumped together in a clear space. No double contours can be seen, neither does this débris stain with hematoxylin-

eosin, methylene blue or mucicarmine. However, moist sodium hydroxide preparations made from the spleen before fixation showed yeast cells, as well as many bacteria.

Adrenal: One adrenal has a large acute lesion with a necrotic center lying at the end just under the capsule. It is filled with organisms and in places where they are most numerous the parenchyma has completely disappeared and only the connective tissue trabeculae remain (Fig. 3). This area is surrounded by an exudate composed chiefly of plasma cells, with numerous lymphocytes and a few large mononuclear cells; fibroblastic and polymorphonuclear cells are rare. The cell columns just peripheral to the cellular reaction show slight evidence of compression in places. Budding forms are very numerous, many more than in the meninges (Figs. 3 and 6). Clear zones surround organisms in areas of necrotic tissue. In the same adrenal another acute lesion has formed around one of the central veins; the lumen of the vessel is filled with red blood cells, the wall densely infiltrated with torulae. Outside the vessel there are large numbers of organisms in necrotic tissue surrounded by a fibroblastic reaction similar to the other. The other adrenal contains a smaller, more acute lesion with very little tissue destruction and a small number of organisms. There is very little cellular reaction around it, but budding forms are very numerous.

Lungs: The lungs contain large dense scars with small, irregular, calcified foci. Surrounding them is fibrous tissue proliferation containing many fibroblastic nodules with giant cells and epithelial cells like those seen in the spleen. Some of the giant cells contain spaces with sharp borders, as if crystals had been dissolved out. Acute bronchitis and small areas of bronchopneumonia are scattered through the lungs. No yeast-like organisms or tubercle bacilli are seen.

Lymph Nodes: Most of the lymph nodes are completely replaced by fibrocaseous material with no active processes present. A large node near the hilum of the spleen is the seat of extensive hyaline degeneration and marked endothelial hypertrophy. There are occasional small tubercle-like structures with and without giant cells in the center, which in places are grouped together but separated by fibrous tissue. Yeast fungi could not be found, but some giant cells contain spaces resembling the residues of organisms like those in the spleen.

Kidney: The epithelium of the kidney is swollen, granular and in places necrotic.

The other organs contained nothing of interest.

Brain and Meninges: Sections for microscopic study were taken from the cerebral hemispheres, corpus striatum, midbrain, pons and cerebellum. They were stained with thionin, mucicarmin and scarlet red. Myelin sheath stains were made after the method of Weil, and nerve fiber stains after Bielschowsky. In addition the meninges were stained with hematoxylin and eosin, with carbolfuchsin for tubercle bacilli, and by Van Gieson's method for connective tissue.

The leptomeninges over the convolutions are slightly thickened and infiltrated with plasma cells. Wherever there is a blood vessel a marked cellular reaction is present around it, and where numerous blood vessels occur close together a granuloma is formed in the meninges. Over the convexity of the brain, where the reaction is less marked, degenerative changes are few; plasma cells predominate and there are many cells with large, oval or elongated, pale staining nuclei and lymphocytes. Giant cells are scattered through the meninges, most often forming the center of a nodule. Considerable connective tissue is shown by the Van Gieson method. Many fat globules can be seen in scarlet red preparations, usually within cells in proximity to areas of necrosis, but not within the areas of marked necrosis.

There is a granuloma at the base of the corpus striatum, arising in the meninges and extending a considerable distance into the brain. In this area are many giant cells and numerous areas of complete degeneration. There are many lymphocytes and polymorphonuclear leukocytes, the former predominating; plasma cells and cells with large, pale, oval or elongated nuclei are fewer than over the convexity of the hemispheres. There is much rather old connective tissue and many new blood vessels in the granuloma. Torulae are scattered through the meninges, often occurring in large groups without evidence of cellular reaction around them (Figs. 4 and 5). The adjacent area of the brain has many dilated blood vessels with marked perivascular lymphocytic and leukocytic infiltration, and many inflammatory cells free in the tissues, of which the polymorphonuclears predominate. Pial funnels greatly thickened by a lymphocytic reaction dip far into the brain.

The granuloma covering the corpora quadrigemina, which is 6 or 7 mm. thick, is composed largely of necrotic material resembling caseation in some areas. The only cells not having degenerative changes are in immediate proximity to the blood vessels. They are chiefly lymphocytes, but polymorphonuclear leukocytes, plasma cells and large mononuclear exudate cells are numerous. Often a vessel will have a thickened wall almost surrounded by organisms, which are surrounded by a thick collar of lymphocytes or leukocytes with degenerated areas peripheral to this.

Most of the blood vessels show proliferation of the adventitia and often contain torulae within their walls; some have groups of torulae in their walls not surrounded by inflammatory cells. Several large vessels present marked endarteritis, the lumen being almost obliterated by a proliferation of endothelium. Torulae are found in the intima of several of these vessels. One space lined with flattened, elongated cells resembling endothelium is filled with organisms.

The ganglion cells of the cerebral cortex are in various stages of degeneration; most of them are swollen and distorted, with a complete chromatolysis of the Nissl substance, and many appear as faint outlines. The astrocytes are increased both in size and in number. The myelin sheaths in the subcortical white matter are greatly thinned out. They are swollen, fragmented, and in places small vacuoles are present. A normal number of myelin sheaths enter the cortex but they are swollen, beaded in appearance and lack continuity. Many mucocytes occur throughout the white matter. The nerve fibers in the white matter are severely broken up, the fragments are beaded and often irregularly shaped globules are the only remnants. There is no fatty degeneration, except in the perivascular inflammatory cells.

Ganglion cells, myelin sheaths and nerve fibers in the corpus striatum show changes similar to those in the cortex. No fatty degeneration is present. The lower part of the lenticular nucleus, adjacent to the granuloma described above, has many dilated blood vessels with marked perivascular, lymphocytic and leukocytic infiltration. Many inflammatory cells are also found free in the tissue, the polymorphonuclears predominating. The ependyma is much thickened and underlying it is a marked proliferation of glia which produces a protrusion of the ependymal lining into villus-like structures (granular ependymitis).

The nuclei of the pons and midbrain are in fairly good condition. Some of the ganglion cells are slightly swollen and others have a somewhat sclerotic appearance. The myelin sheaths are less damaged than in the subcortical white matter. Mucocytes, which are pink-stained with mucicarmin, are numerous. There is a triangular area of incomplete softening with the base on the surface of the pons and the apex inside. It is filled with large scavenger cells and invaded by many new blood vessels. The border line is sharp. The parenchymatous elements about the periphery are fairly well preserved and the microglia are increased. Myelin has completely disappeared from this infarct. The subarachnoid space surrounding the midbrain and pons is completely obliterated.

Complete chromatolysis of Nissl's substance has occurred in the ganglion cells of the dentate nucleus. Throughout the granular layer of the cerebellum are small foci where these cells have dropped out. All of the Purkinje cells show degenerative changes; many are rounded or distorted and appear as faint outlines or shadows. In one area there is a complete falling out of the granular and Purkinje cell layers of one-half of a folium and the adjacent half of the neighboring folium. In this area the glia are somewhat increased. The scarlet red stain discloses many fat globules in the cortex of the adjacent halves of the two folia, extending centrally as far as the granular layer, with only an occasional droplet within it. The myelin sheaths in the white matter are greatly thinned out; they are swollen, fragmented, and in places small vacuoles are present. The myelinated fibers entering the folia are sparse and no fine fibers can be seen entering the granular layer. Using Bielschowsky's method, the nerve fibers in the cortex of the cerebellum are seen to be fairly well preserved. In the white matter they are markedly fragmented, and the fragments are beaded or appear as irregularly shaped globules.

There are two areas of softening about 1 mm. in diameter, one being in among the ganglion cells of the dentate nucleus, and the other in the white matter enclosed by the arms of the nucleus. The latter has in it several large giant cells containing torulae; these lie in the midst of a large collection of polymorphonuclear leukocytes which are surrounded by a thick ring of cells with large, oval, pale nuclei. Scattered through this lesion are similar pale nuclei which have assumed a more rounded or a more elongated form, as well as

numerous torulae, plasma cells and lymphocytes. The other area of softening is different in many respects. Several blood vessels with a lymphocytic infiltration about them enter it. The cellular reaction is composed largely of glia and cells with pale elongated nuclei; polymorphonuclear and plasma cells are rare and no torulae are present. A long, narrow zone of degeneration extending from the cortex centrally through the white matter is revealed by the presence of many fat globules, most of which are within cells.

In one area in the dentate nucleus are numerous groups of small, densely and evenly staining round bodies from pin-point size to 2 microns in diameter, which stain purple with thionin. They are usually discrete but often appear to be budding. They generally occur in double file along a straight or sinuous course, and the flattened endothelial cells of a capillary can usually be identified along the route. They are most probably minute torulae within capillaries.

The Organism: Various stains were used and although the organisms took many of them well, they were difficult to identify in the tissues unless present in large numbers. In looking for a differential stain, those for mucin were considered because of the gelatinous substance produced by torula in the meninges. Bailey and Schaltenbrand have demonstrated that the clear, non-staining substance which occurs in acute swelling of oligodendroglia is in reality a mucin-like substance. This was shown by Grinker and Stevens to be a specific type of regressive change found in no other glia, and represents the same process as mucoid degeneration. Torula stains the same color with mucicarmin, but the morphology is so different that no confusion will arise in the differentiation from mucocytes, although the latter are numerous in the brain in this case. By this method the organisms can be easily found with the low power objective of the microscope: they are pink, the cell nuclei brown and cell cytoplasm yellow. Goto obtained a somewhat similar result using Best's carmin stain.

Stained by this method the organism under the lower power of the microscope is a round or oval, somewhat refractile body with a pale staining center and a deeply staining pink wall. Under the oil immersion objective the small forms have a homogeneous center and a single line composing the wall. Often the medium sized and large forms have a double-contoured wall. When the inner one is brought

Table II

Cultures of Yeast-Like Organisms from Spleen *

Sugar Concentration as Indicated

Strains		1% Dextrose	0.5% Fructose	0.5% Galactose	0.5% Inulin	0.5% Lactose	0.5% Mannose	1% Sucrose	0.5% Maltose	0.5% Xylose
Non-pigmented	3	Sl⊕(1)	Sl +(1)	....	....	....	Sl +(1)	....	....	....
	4	Sl⊕(1)	Sl +(1)	....	....	....	Sl +(1)	....	....	....
	6	Sl⊕(1)	Sl +(1)	....	....	....	Sl +(1)	....	....	....
Pigmented	7	Sl +(7)	....	....	....	....	....	....	....	....
Known stock	Torula rosa	Sl +(14)	Sl +(14)	....	....	....	Sl +(14)	Sl +(14)	....	....
	Saccharomyces cerviciae	⊕(1)	Sl +(1)	Sl +(1)	....	....	Sl⊕(1)	Sl⊕(1)	Sl +(14)	....
	Oidium lactis	....	....	....	....	....	....	....	....	....
	Blastomycetes dermatitis	....	....	....	....	....	....	....	....	....

Sugar Concentration of 2.5 Per Cent

		Dextrose	Fructose	Galactose	Inulin	Lactose	Mannose	Sucrose	Maltose	Xylose
Non-pigmented	3	⊕(2)	⊕(2)	....	....	....	⊕(3)	....	....	....
	4	⊕(2)	⊕(3)	....	....	....	⊕(3)	....	....	....
	6	⊕(2)	⊕(2)	....	....	....	⊕(3)	....	....	....
Pigmented	7	Sl +(7)	Sl +(12)	....	....	....	Sl +(14)	....	....	....
Known stock	Torula rosa	Sl +(7)	Sl +(7)	....	....	....	Sl +(7)	Sl +(12)	....	....
	Saccharomyces cerviciae	⊕(2)	⊕(3)	+(3)	....	....	⊕(3)	+(2)	⊕(2)	....

* + = acid ⊕ = acid and gas Sl+ = slight acid Sl⊕ = slight acid, one bubble of gas ( ) = figure in parentheses represents day reaction first appeared

sharply into focus the outer appears as a less deeply staining, refractile ring. Pink spicules radially arranged, with a broad base attached to the outer wall of the organism, and a pointed end are present on many of them (Fig. 5). In the tissues these yeast-like bodies are often surrounded by a wide clear zone, especially when in groups or in tissue undergoing regressive changes (Figs. 3, 5 and 6). The distal ends of the spicules usually extend out to the periphery of this clear zone but have not been seen to reach beyond it. This effect is most probably due to the action of the fixative.

The centers of the medium sized and large forms have several variations; some are homogeneous like the small ones; others are homogeneous except for the presence of two to five irregularly shaped, clear spaces, or non-staining inclusions. Many, however, have the entire portion within the wall composed of a tan, flocculent material (Figs. 5 and 6). This may be evenly distributed; it may contain in it clear spaces, or when small in amount form a ring just medial to and touching the inner wall of the organism.

Often a black, chromatin-like substance lies within the organism, which gives it the appearance of having a nucleus.

Most of the torulae occur free in the tissues but are sometimes found within giant cells (Fig. 13). Here they vary much in the depth of the stain taken. Some are deep pink, others in the same giant cell are colorless. There can be no doubt about the identity of the latter because of their characteristic size and shape, and some even have a double-contoured wall. This is important to note, because the inclusions in the giant cells of the spleen, lung and perisplenic lymph node may be of this nature, though it is true that no double-contoured forms are seen.

Reproduction is by budding, occurring usually in medium sized and large forms (Figs. 3, 5, 6 and 7). All stages are seen from a small bud-like projection from the circumference of the organism to dumb-bell forms where the mother and daughter cells are of equal size. Finally the two bodies separate, though occasionally they continue to be connected by a pink-staining band. Usually the daughter cell breaks off when it reaches one-half to two-thirds the size of its parent.

Budding forms are numerous in the lesions in the adrenals, much less frequent in the meninges. No budding has been seen in

giant cells; the colorless zone surrounding the bodies, so frequently present in the extracellular ones, is occasionally found here. No spores, mycelia or hyphae are seen.

Bacteriological Examination *

Method of Isolation: Moist sodium hydroxide preparations of the spleen showed under the microscope yeast cells, as well as many bacteria. Small blocks were removed aseptically from the tissue and streaked on the following media: Sabouraud's dextrose agar plates, carrot cylinders, Corper's glycerinated potato cylinders, Petroff's, and Hohn's egg medium. The cultures were made in duplicate, one set being incubated at room temperature and the other at 37° C.

A part of the tissue was ground in a sterile mortar and emulsified in normal saline. One half of this was heated to 53° C for twenty minutes to kill off the bacteria. Two rats and two mice were then inoculated with each emulsion. The mice died with evidence of bacterial infection, but no yeast-like organisms were recovered. The rats remained normal.

Yeast-like organisms grew out in Sabouraud's agar, carrot cylinders and the egg medium of Petroff and of Hohn (see Húth). By repeated subculture on Sabouraud's agar, pure cultures were obtained from the carrot cylinder. Two varieties of yeast were isolated, one producing no pigment and one producing a yellow pigment.

Cultural Characteristics: (a) Strain Producing No Pigment: On Sabouraud's agar the growth is white and shiny. The colonies are discrete, spreading slightly with age (Fig. 10). The cells are round and quite uniform in size and shape. Budding forms are quite numerous in young cultures. Fermentation tests were made using both 0.5 per cent and 2.5 per cent concentrations of the carbohydrates. The higher concentration of sugar seemed to hasten the reaction and cause a definite amount of gas to be formed in some of the sugars (Table II). In this, acid and gas were formed in dextrose, fructose and mannose. There was no reaction in galactose, inulin, lactose, sucrose, maltose or xylose at the end of fourteen days.

(b) Strain Producing Yellow Pigment: On Sabouraud's agar the growth is a light yellow at first, becoming more deeply pigmented as

* I was assisted in the bacteriological work by Miss Elizabeth Petran and Miss Bertha Kaplan of the Department of Hygiene and Bacteriology.

the culture ages. The colonies spread slightly. There is a variation in the size and shape of the cells. Budding forms are numerous in young cultures. With carbohydrate concentration of 2.5 per cent, dextrose was fermented on the seventh day with production of acid without gas. Fructose and mannose showed a slight amount of acid on the twelfth and fourteenth days respectively. Neither acid nor gas was formed in any of the other sugars tested (Table II). With 0.5 per cent concentration of carbohydrate similar reactions were obtained, except that fermentation of dextrose did not appear until the fourteenth day.

Animal Pathogenicity: Rats and mice were inoculated intraperitoneally with pure cultures of each of these strains. The mice died during the third week after inoculation. Autopsies were performed and cultures made from the tissues but no yeast-like organisms were recovered.

The rats remained well and were sacrificed one month after inoculation. The rats inoculated with the strains producing no pigment (*a*) were normal so far as could be determined, and cultures made from the liver, spleen and kidneys were sterile. The rats inoculated with the strain producing yellow pigment (*b*) showed white patches on the spleen and a few on the liver. These white patches were similar to those described by Tanner and Dack in rats inoculated with yeasts isolated from sore throats, but yeast could not be isolated in cultures of these organs. Histological sections of the spleen contained nothing which could be definitely identified as a yeast.

Two guinea pigs were injected in the groin with cerebrospinal fluid Jan. 10, 1931, a short time before the patient's death. One died February 19, and autopsy revealed exudate in both pleural cavities and some consolidation of the lungs. Some elements in the microscopic sections might be considered yeast, but none could be cultured from this exudate or any of the other viscera, though bacteria were numerous. The second pig, which had been in good health, was killed April 18 and cultures made on Sabouraud's medium, Heinaman's potato medium and in dextrose broth from the brain, liver, spleen, kidney, lung, peritoneal cavity and heart's blood. All organs appeared normal. Pure cultures of a budding, yeast-like organism were recovered from lung and kidney. These organisms were oval and stained very faintly with mucicarmin, but a deep

violet with the Gram-Weigert stain. These were transferred to 2.5 per cent dextrose, lactose, sucrose, maltose, mannose, galactose, inulin, fructose and xylose; acid and gas formed in dextrose, mannose, and fructose in twenty-four hours, but no change appeared in the others in two weeks. These cultures were also put into 1 per cent dextrose, lactose and saccharose, and 0.5 per cent maltose, mannose, inulin, xylose, fructose, rhamnose, raffinose, ducitol and galactose, with the formation of acid and gas within twenty-four hours in the dextrose and fructose. No reaction appeared in the other tubes on prolonged standing. Cultures from the lung and the kidney produced identical reactions. Torulae were not found in microscopic sections of lung, kidney or other organs. This strain was translucent to white, at first becoming brownish with age (Fig. 12). It fermented the same sugars as the pigment-producing one from the spleen. On April 24, two guinea pigs and two rats were injected intraperitoneally with cultures from the lung. One of the rats died September 11, the other animals were sacrificed the same day and cultures made of the brain, lungs, kidneys and spleen on Heinaman's potato medium and in dextrose broth. An oval, budding organism morphologically like the one injected was recovered from the brain and spleen of the rat that died, and from the spleen of one of the guinea pigs. All of the tissues appeared normal.

CASE 2. * Mrs. F. H., aged 48 years, entered the University of Iowa Hospital, Feb. 20, 1929, complaining of headache, weakness and coldness of the extremities, inability to speak, and difficulty in swallowing. Her family history was of interest, in that one child died of meningitis. The patient had pneumonia in 1925.

Present Illness: Began Nov. 15, 1928, three months before entering the hospital, with headache, pain in back of the neck and vomiting. Five days later she complained of weakness and coldness of the extremities, more on the right than the left. November 22 she became unable to speak and attempts to eat produced attacks of strangling and coughing. This continued and she was unable to take anything but fluid after this symptom appeared, and consequently lost 70 pounds in weight. About one week before admission to the hospital her husband noticed she was acting queerly; she would sit huddled up in bed with her mouth hanging open, apparently unable to keep it closed. All of her symptoms continued and she was brought to the hospital.

Physical Examination: Showed a markedly emaciated, white woman, sitting doubled up in bed, mourning and shaking her head, mouth hanging open, unable

* Through the courtesy of Drs. G. H. Hansmann, C. Van Epps, and C. F. Obermann of Iowa City I have been able to study this case, which has features not present in my own.

to speak, but pointing to the right side of her chest as though it hurt. The temperature was 101.8° F, pulse 140, respirations 48. The teeth were in poor condition, mouth dirty, tongue parched, lips covered with sores and breath foul. She could close her mouth voluntarily but seemed unable to keep it closed. The chest was resonant throughout but the breath sounds could barely be heard over the right lung. The heart sounds were clear and distinct at the apex but distant elsewhere; the heart did not appear enlarged. Ophthalmoscopically the blood vessels were moderately engorged, disk margins blurred and there was slight papilledema. Pupils were equal, reacted to light and accommodation and the extraocular movements were normal. No facial weakness. The tongue could be protruded only a short distance but it appeared in the midline. All extremities were rather weak but there was no paralysis. A coarse, slow tremor of the hands was present and the muscles of the arms were rather stiff. Sensation and reflexes in upper and lower extremities were normal. A bilateral unsustained ankle clonus was obtained. There was a little stiffness of the neck and a slightly positive Kernig's sign.

Laboratory Data: A lumbar puncture was made and 96 cells found, 84 of which were lymphocytes and 12 polymorphonuclear leukocytes. A smear was made for tubercle bacilli but none was found. A culture of the fluid yielded *Staphylococcus aureus* and a diphtheroid bacillus.

Course in Hospital: Four days after admission her condition grew rapidly worse, the extremities became cold and cyanotic and large bubbling râles could be heard in the chest. Postmortem roentgenogram of the chest showed an extensive pneumothorax on the right side.

Autopsy Report

The autopsy was performed by Drs. E. D. Peasley, J. W. Budd and C. F. Obermann thirteen hours after death.

The only significant findings were in the lungs and the central nervous system. The left pleural cavity contained a small amount of clear, straw-colored fluid. When the right pleura was punctured a considerable amount of foul-smelling gas escaped under pressure. About 400 cc. of grayish, purulent fluid was present and the lung had collapsed so that it occupied about one-fifth of the cavity. An abscess 1 cm. in diameter could be seen just beneath the pleura near the base of the inferior lobe of the right lung on the anterior lateral aspect. The bronchi contained mucopurulent exudate which completely occluded many of them; there was marked bronchitis and tracheitis. The left lung appeared normal, except for a few healed lesions in the serosa.

Brain and Meninges: The leptomeninges of the brain were thickened and had a milky, grayish appearance, most marked around the interpeduncular region and fissure of Sylvius. The blood vessels were markedly congested. The convolutions were normal.

The brain was cut into thin coronal sections which revealed numerous cystic cavities containing a gelatinous material. They were found (1) in the basal ganglia of both hemispheres, varying in size from 1 to 8 mm. in diameter (Fig. 2); (2) the right cerebellar hemisphere at about the dentate nucleus, 1 to 3 mm. in diameter (Fig. 14); (3) the cortex of the right frontal lobe on its ventral aspect, and (4) the pons in the vicinity of the substantia nigra. There was moderate hydrocephalus.

The meninges of the spinal cord were thickened and the blood vessels were congested. Several white, flaky patches were seen in the meninges. The cord appeared normal externally and on section.

Microscopic Examination

Lung: Microscopically the greatest changes are in the right lower lobe of the lung, where there is an extensive pneumonic process of the lobular type. Between the patches of pneumonia are many large abscesses filled with polymorphonuclear leukocytes with relatively little fibroblastic reaction. Several large foreign body giant cells are present, but no yeast organisms can be found although they were isolated by culture from the pleural exudate.

Meninges: Microscopically there is a chronic inflammatory process in the meninges. The exudate is made up largely of lymphocytes and plasma cells, with a few polymorphonuclear leukocytes. Giant cells are quite numerous; the nuclei in some are arranged about the periphery and in others are grouped together in the center of the cell, varying from ten to twenty in number. Torulae are found scattered through the meninges and within giant cells; they vary considerably in size. Budding forms are seen occasionally. Perivascular reaction is much less prominent than in Case 1.

The cysts have a characteristic appearance (Fig. 9). A typical one appears as a cavity with practically no reaction around it. Often it is multilocular, being divided into compartments by strands of necrotic brain tissue running from one wall to another. The wall in many of the cysts appears compressed, but not sufficiently to account for its size by expansion alone, due to the material produced by the organism. Large numbers of organisms lying free and in necrotic material near the periphery are present. Those lying in areas of necrotic material are surrounded by clear zones resembling

a halo. Around a large cavity are often numerous small cavities, each containing one or more organisms. The organisms are very large, often double-contoured with rare budding forms (Fig. 15). Numerous convexoconcave and biconcave forms, like those shown by Freeman in his comparative study of this condition, are seen. Other forms have three concavities visible. Several cysts are seen which contain a moderate sized blood vessel; in all of these, and in a few others where no vessel can be identified, there is some round cell reaction.

The spinal meninges resemble those of the brain except that the organisms are fewer. No lesions are found in the spinal cord.

Cultures were made at autopsy with the following results: (*a*) blood: *Streptococcus hemolyticus*; (*b*) empyema: a yeast-like organism, a streptococcus, and *B. proteus*; (*c*) lung abscess: *Staphylococcus aureus*, a streptococcus, and *B. proteus*; and (*d*) brain: *Streptococcus hemolyticus* and *B. proteus*. Unfortunately the yeast grown from the pleural exudate was not saved, so no studies were made of it.

Discussion

Torula is distributed widely in nature, having been cultivated from wasp nests, the stems of many plants and grasses, the bodies of numerous insects, and from pickle bran (Buchanan, Duggar, Stevens). It has been found in milk (Klein) and in canned butter (Rogers). It seems that all types are originally non-pathogenic, becoming pathogenic only under suitable conditions. The low pathogenicity is borne out clinically by the fact that most cases are recognized only when they attack the central nervous system.

Freeman divided the lesions of the brain into three general orders: meningeal, perivascular and embolic. The perivascular is frequently associated with the meningeal. Except in Ball's Case 1, meningitis was present in all of those with cystic cavities, which are considered by Freeman to be embolic phenomena. Cystic cavities were reported by Ball, Benda, Bettin, Flu and Woensdregt, Freeman and Weidman, von Hansemann, Pierson, Rusk and Farnell, Smith and Crawford, Stoddard and Cutler, Stone and Sturdivant, Weil, White, and Williams. Cases with meningitis without cysts were recorded by Evans, Goto, Hall and Hirsh and Mock, Hansmann, Hirsh and Coleman, Lynch and Rose, Massee and Rooney, McKendree and

Cornwall, Rappaport and Kaplan, Shapiro and Neal, Semerak (two cases), Sheppe, Stoddard and Cutler, Swift and Bull, Türck, Versé, Wilhelmj, Wortis and Wightman. Case 1 of this report belongs to the latter group and Case 2 to the former. In five, the diagnosis of meningitis was made by detecting the organism in the cerebrospinal fluid (Evans (two cases), Lynch and Rose, Shapiro and Neal, Wortis and Wightman), an autopsy not having been performed. Stoddard and Cutler, and Freeman in his comparative study and also in his paper with Weidman, noted degenerative changes in the ganglion cells, increase of neuroglia and myelin sheath degeneration; Rusk found the nerve cells normal. Most observers have completely ignored these elements, confining their attention entirely to the organisms and the more prominent changes produced by them. In addition, my first case showed an infarct in the pons and a marked atrophy of the cerebellum.

The portal of entry is probably the respiratory tract in most cases. Torulae were found in the lungs of several cases (Hall, Hirsh and Mock, Pierson, Rusk and Farnell, Sheppe, Stone and Sturdivant, White, and Williams) of which Sheppe's case has the distinction of being the only one without clinical signs of cerebral involvement. Many others had pulmonary disease of another nature: pulmonary tuberculosis (von Hansemann, Lynch and Rose, Rappaport and Kaplan, Stoddard and Cutler, Versé); tuberculous bronchial lymph nodes (Hansmann); terminal pneumonia (Massee and Rooney, Pierson, Stoddard and Cutler, Smith and Crawford). Torulae were cultured from the upper respiratory tract in three cases (Evans, Rappaport and Kaplan, Türck), tonsillitis preceded the illness in three (Freeman and Weidman, Jones, Sheppe) and otitis media in three (Bettin, Stoddard and Cutler, and Wilhelmj). Alvarez described a red torula with cultural characteristics like the others, grown from a patch of reddish hair-like filaments on the tongue, which produced symptoms by tickling the uvula; there were no systemic symptoms. Berghausen cultured a torula from an ulceration of the tongue which developed following an injury; this was associated with pleurisy and X-ray evidence of consolidation of the lungs, and a palpable spleen. The patient died of inanition. Curetting from nodules in the pharynx revealed the organism to Jones, in a patient in whom all of the symptoms were local for months but later became systemic.

The finding of torulae in the gasserian ganglion in four cases (Hall, Hirsh and Mock, Hirsh and Coleman, and Semerak (two cases)) suggests direct extension from the pharyngeal structures. Although most cases have been reported as meningo-encephalitis, and a few as localized abscesses (Brewer and Wood, McGehee and Michelson) and infections of the nasopharynx, many were generalized (Hall, Hirsh and Mock, Pierson, Rappaport and Kaplan, Rusk and Farnell, Sheppe, Stone and Sturdivant, Versé, White, Williams, and Watts' Case 1). After the central nervous system, the lung, spleen, kidney and adrenal each contain the organisms most frequently. In contrast to oidiomycosis and coccidioidal granuloma, a skin lesion has been present in only one case (Rappaport and Kaplan) and a bone lesion in one (Brewer and Wood). In the latter the spinous processes and laminae were eroded but no torulae were found in the bone itself.

Dissemination frcm the respiratory tract may be by the blood stream or lymphatics. Organisms in the gasserian ganglion of four cases suggest lymphogenous infection. Spitzer's experiments support this view; he produced an ascending inflammatory neuritis spreading up to and implicating the gasserian ganglion by injecting *Abrus precatorius* into the dental pulp of dogs. The infarct in the pons, torulae in the intima of meningeal vessels producing an endarteritis, and minute torulae in capillaries of the cerebellum of Case 1 add weight to the theory that cystic cavities in the deep white, or gray matter of the brain are embolic phenomena. Here by embolic phenomenon is meant not the occlusion of a small vessel with resulting ischemic liquefaction, but the passage of torulae by the blood stream and the deposition with the production of a cyst by the organisms, by lysis, or by expansion.

The cerebrospinal fluid findings are what one would expect in chronic meningitis. The sugar has been reduced in all cases in which it was noted to about the same degree found in tuberculous meningitis, as shown in a review by Watts and Viets. The chloride content, measured as sodium chloride, was reduced to the unusually low level of 464 mg. per 100 cc. This is even lower than occurs in tuberculous meningitis, and Fremont-Smith in his comprehensive review records no figure as low. The colloidal gold curve has been noted in very few instances, but in three it was distinctly paretic in type (Hansmann, Wortis and Wightman, and Watts). Several instances of the non-specific character of the curve are given by Watts and

Mixter in spinal epidural granuloma. Seven cultures made of the cerebrospinal fluid on blood agar and Rosenow's medium were negative after three days. Of twenty-three cases in which cultures were attempted, growth was obtained in seventeen; in one of these, cultures were negative in the early stages of the disease. In six no growth was obtained, in spite of the fact that the organism was seen on smear of the fluid.

Harrison's classification of the torulaceae on physiological characteristics appears to be the best to follow and is recommended by Henrici who says: "He retains the genus Mycotorula of Will for those forms producing rudimentary mycelia. The remaining species are grouped according to pigment production into the genera — Rhodotorula with red pigment, Chromotorula with pigment other than red, and Torula with no pigment. Torula and Mycotorula are further divided into groups according to their sugar fermentations."

Group A. No acid or gas in any sugar.
Group B. Slight acid in dextrose, mannose, fructose or galactose.
Group C. Slight acid with or without trace of gas in dextrose, mannose, fructose or galactose, and saccharose.
Group D. Marked acidity and gas in dextrose, fructose, or galactose, and mannose.
Group E. Marked acidity and gas in dextrose, mannose, fructose, galactose and saccharose.
Group F. Marked acidity and gas in dextrose, mannose, fructose, galactose, saccharose and raffinose.
Group G. Marked acidity and gas in dextrose, mannose, fructose, galactose, saccharose and maltose.
Group H. Marked acidity and gas in dextrose, mannose, fructose, galactose, saccharose and lactose.
Group I. Marked acidity and gas in dextrose, mannose, fructose, galactose, saccharose, lactose and inulin.

According to this classification the strain producing no pigment falls into *Group D* of torula. The strains producing pigment appear to be chromotorula.

Torula infection should be considered in the differential diagnosis by clinicians where symptoms of increased intracranial pressure of unknown etiology, prolonged chronic meningitis, and chronic pulmonary conditions are present. The organism should be looked for in the cerebrospinal fluid and the sputum. Likewise, pathologists should be more guarded in calling all chronic meningeal and pulmonary disease tuberculosis when the tubercle bacillus is not found.

No treatment is known which affects the course of the disease, but Stone and Sturdivant have inhibited the growth of the organism *in vitro* by the use of gentian violet, gold sodium thiosulphate, and X-ray therapy.

Summary

1. Two cases of torula infection are presented. In the first the infection was generalized but the symptoms were almost entirely cerebral. A remarkable collection of pathological changes were present in the brain: diffuse meningitis, granulomas in the meninges, marked endarteritis and proliferation of adventitial elements of the meningeal vessels, an infarct in the pons, areas of softening and focal disappearance of the granular and Purkinje cells in the cerebellum, diffuse ganglion cell changes, increase of neuroglia, nerve fiber destruction, myelin sheath damage, and encephalitis by extension in the striate body. The second case falls into the group which Freeman considers embolic phenomena.

2. Mucicarmin was found to be an excellent differential stain, not only making it easy to identify the organism by its distinctive color, but bringing out details of structure not hitherto recorded.

3. Two strains of yeast-like bodies were isolated: the one producing no pigment falls into the torula group; the one producing pigment appears to be chromotorula. The organism was non-pathogenic for guinea pigs, rats and mice.

4. The respiratory tract is probably the portal of entry in most cases. The infarct in the pons, endarteritis of numerous meningeal vessels with torula in the intima, and softenings in the cerebellum in Case 1 add weight to the theory that cystic cavities in the deep white and gray matter are embolic phenomena and dissemination is by the blood stream.

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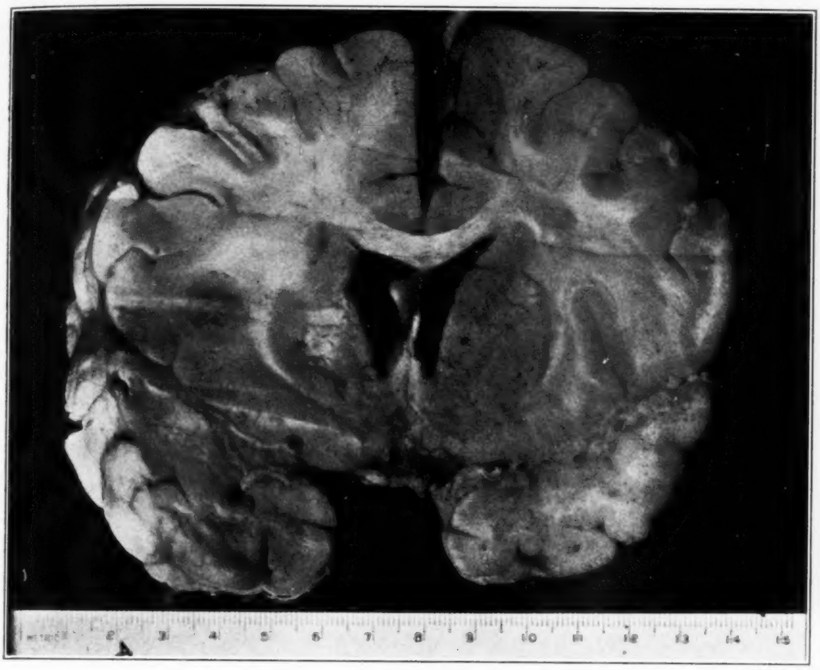
DESCRIPTION OF PLATES

PLATE 29

FIG. 1. Case 1. The pia-arachnoid is thickened and there are many granulomas in the Sylvian fissure.

FIG. 2. Case 2. Cystic cavities 1 to 4 mm. in diameter are seen in both lenticular nuclei.



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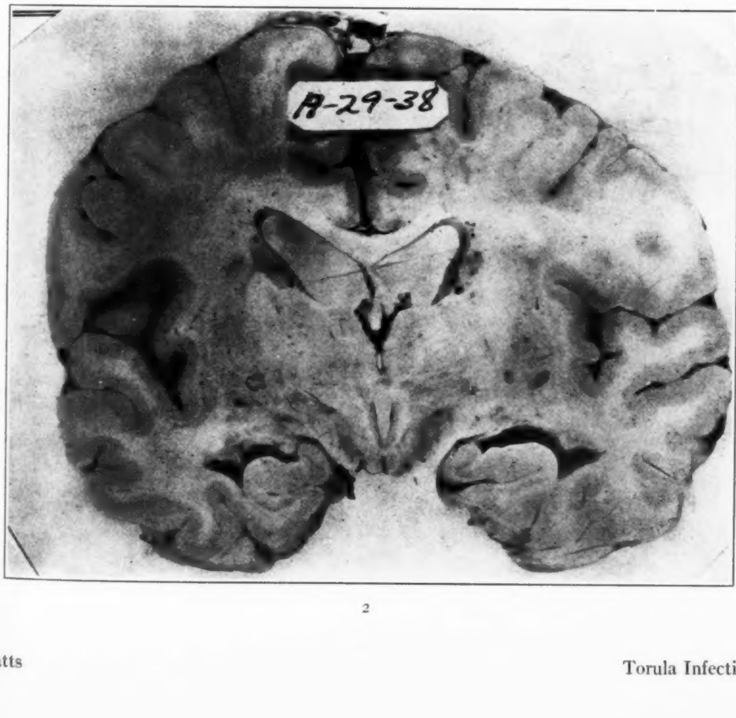

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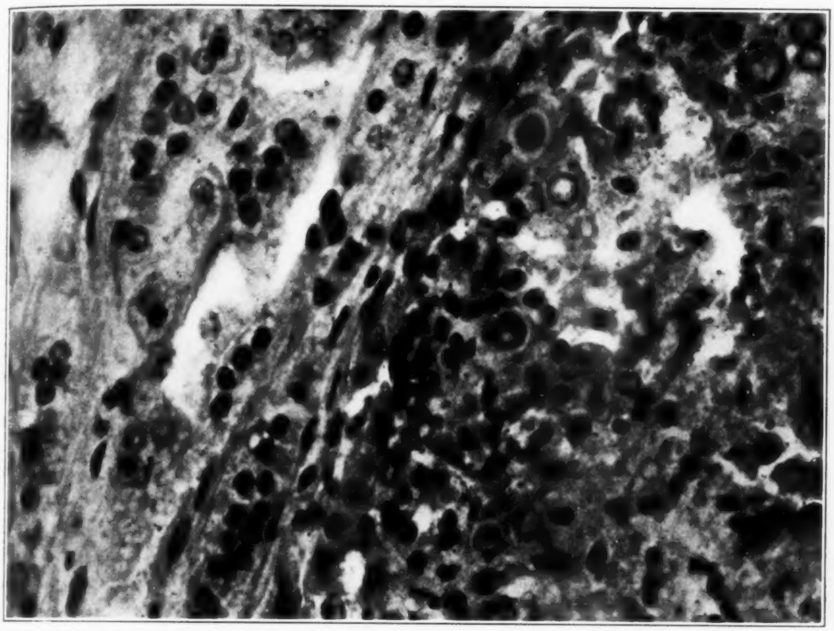
Plate 30

Fig. 3. Case 1. An abscess containing many torulae lying in necrotic material is shown on the right, and normal adrenal on the left. The organisms vary considerably in size; some are surrounded by clear zones. Mucicarmin stain. × 600.

Fig. 4. Case 1. Section through a granuloma in the meninges with numerous torulae and a few giant cells near the center surrounded by a fibroblastic reaction. Mucicarmin stain. × 600.



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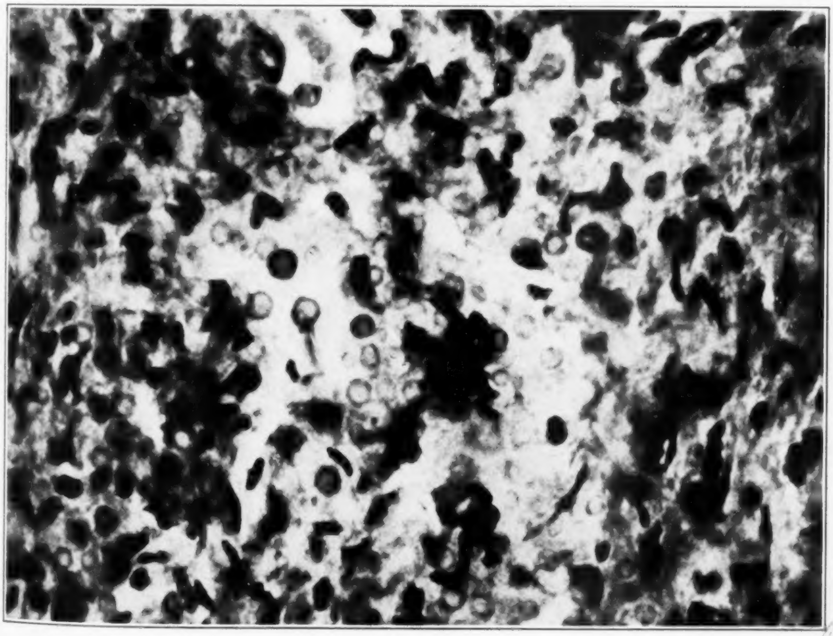

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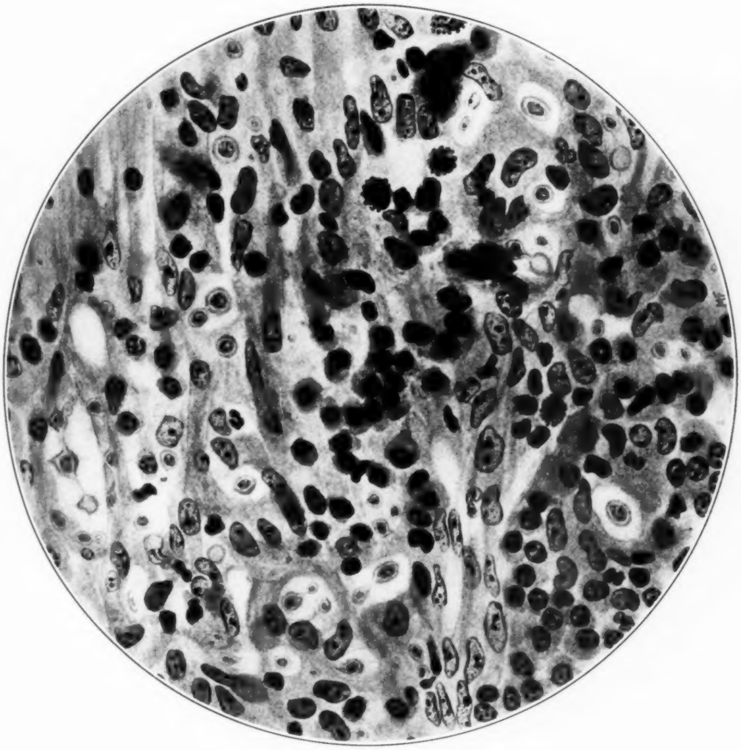
Plate 31

Fig. 5. Case 1. Drawing of Fig. 4 at a higher magnification. Plasma cells and cells with large pale staining nuclei predominate, and numerous lymphocytes are present. Organisms budding, with double contours, having spicules, surrounded by clear zones, and with nuclear-like material are shown. Mucicarmin stain. × 1000.

Fig. 6. Case 1. Drawing of an abscess in the adrenal. Details of the structure of the organism are shown. Mucicarmin stain. × 1000.

Fig. 7. Case 1. A budding torula in the adrenal. Mucicarmin stain. × 1000.



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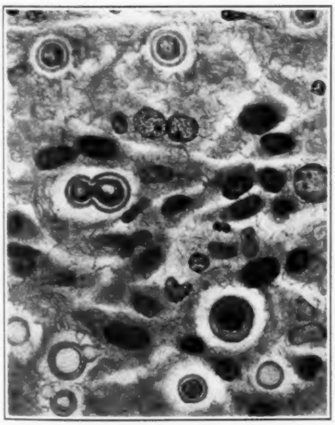

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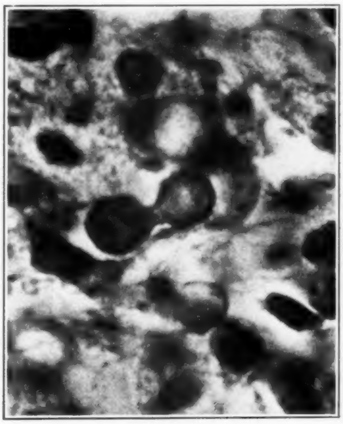

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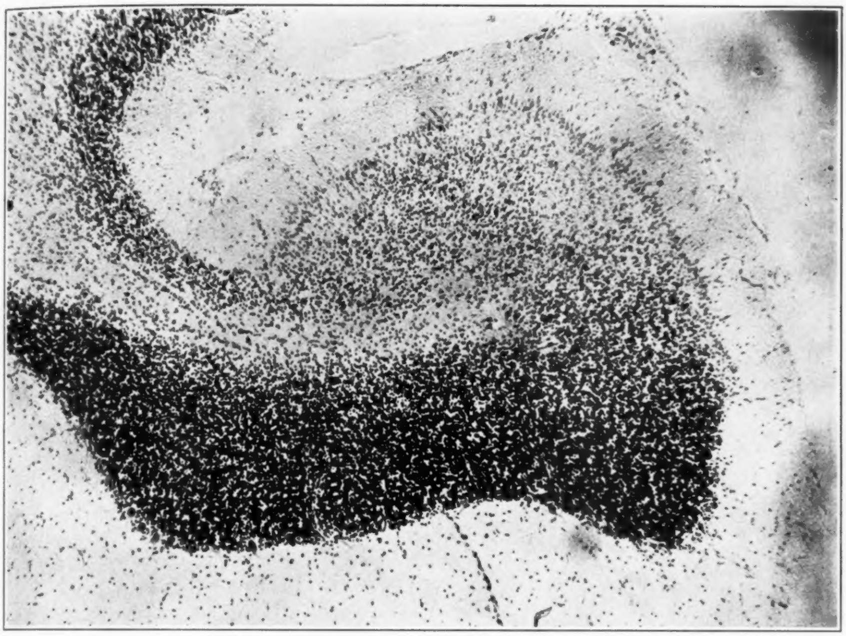
Plate 32

Fig. 8. Case 1. There is an absence of the Purkinje and granular cell layers of the upper half of the folium; the lower half is almost normal. Thionin stain. × 60.

Fig. 9. Case 2. Cystic cavities in the lenticular nucleus with very little inflammatory reaction about them contain many organisms. The black angular material is débris. Hematoxylin and eosin stain. × 60.



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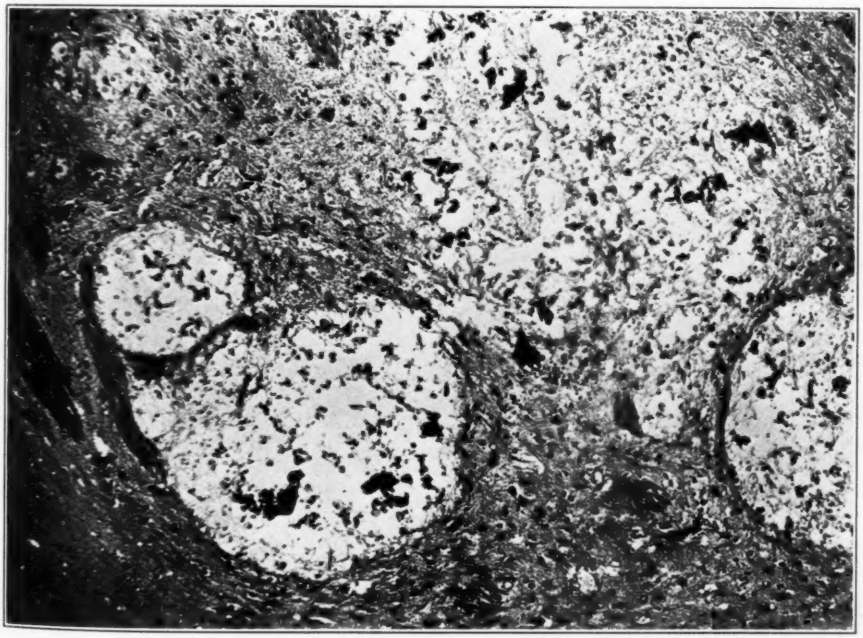

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Plate 33

Fig. 10. Case 1. Superficial and deep colonies of the non-pigmented strain cultured from the spleen. Actual size.

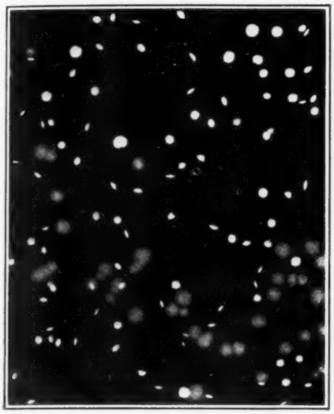
Fig. 11. Case 1. A tubercle-like nodule and blood vessels with exudate about them on the frontal pole.

Fig. 12. Case 1. Colonies of the pigmented strain of torula isolated from the lung of a guinea pig injected with cerebrospinal fluid. Actual size.

Fig. 13. Case 1. Organisms within and without a giant cell in the meninges. Mucicarmin stain. × 600.

Fig. 14. Case 2. Cystic cavities near the dentate nucleus of the cerebellum. Actual size.

Fig. 15. Case 2. Yeast-like organisms with double contours in a cystic cavity in the lenticular nucleus. Hematoxylin and eosin stain. × 1000.



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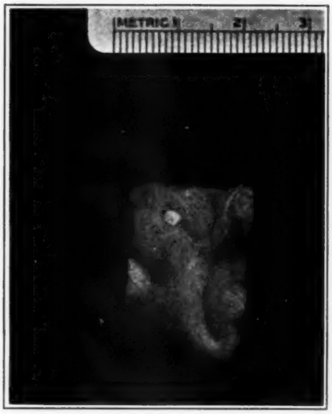

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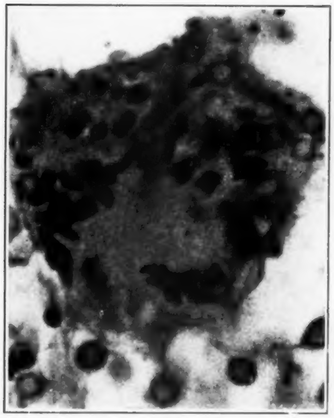

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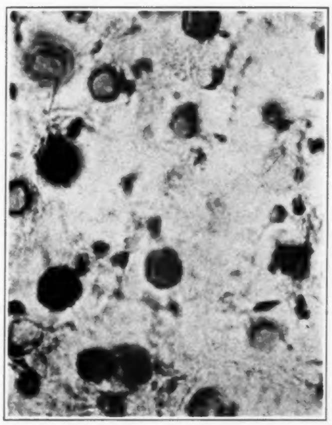

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STUDIES OF EXPERIMENTAL MUSCLE DEGENERATION *

I. FACTORS IN THE PRODUCTION OF MUSCLE DEGENERATION

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The work under discussion deals with a type of striated muscle change known variously as Zenker's degeneration, waxy degeneration, Zenker's hyalin, Zenker's necrosis, vitreous degeneration and hyaline degeneration. Zenker,[1] in 1864, was the first to describe it thoroughly in his classical monograph, using material obtained during the great Dresden typhoid epidemic of 1859–1862.

Zenker described the change which is found most frequently in the adductors of the thigh and the recti abdomini in typhoid fever as consisting in the "conversion of the contractile substance of the primitive bundle to an entirely homogeneous, colorless, strongly wax-like refractile mass, with complete disappearance of the cross-striations and destruction of the muscle nuclei, while the sarcolemma is retained." He named it waxy ("wachsartige") degeneration. Zenker described the gross and microscopic appearance of the fibers and noted the spottiness of the change, the fragility of the fibers, the gross ruptures with hemorrhage and the microscopic separation of contractile material within the sarcolemma sheath. He believed that this change was non-inflammatory in origin, but was due to the forcing in of albuminous substances from outside the fiber because of trophic nerve disturbances from spinal cord damage.

Waxy degeneration of skeletal muscles was observed by Zenker not only in typhoid fever, but also in tetanus, cholera, scarlet fever, and possibly in typhus fever and miliary tuberculosis. Subsequent observers have reported its appearance in various infections such as trichinosis,[2, 3] influenza and pneumonia,[3-7] tetanus,[3, 8, 9] and in diverse conditions such as anaphylaxis,[10, 11, 12] in the neighborhood of malignancies,[12] and in epilepsy.[13]

Von Recklinghausen (cited by Beneke[14]) was the first to consider waxy degeneration not as an entity but merely as one type of the

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general group of hyaline degenerations of cell protoplasm. Nesti [15] was led, as the result of animal experimentation, to a similar conclusion, that is, the waxy change is merely an end stage of an albuminoid metamorphosis, granular and hyaline changes being earlier stages. Beneke's experimental work led him to the same conclusion.[14]

The present work was undertaken to consider whether or not the classical waxy degeneration as described by Zenker is a true pathological entity, and to study some of the factors involved in its production.

Experimental

Healthy, half- or full-grown rabbits were used for most of the experiments. A few white mice were used for bacterial injections and two dogs for study of the effect of high body temperatures. Except when otherwise stated, the animals were fed an ordinary mixed diet. Four main types of muscle injury were employed:

Group 1. Physical Injury: Ligation of the arterial blood supply, mechanical trauma, artificial hyperpyrexia and freezing.

Group 2. Chemical Injury: Lactic acid injections; *in vivo* injections into the arterial blood supply to the muscle, intravenous injections, or direct injections into the muscles themselves; *in vitro* dropping of muscle slices directly after removal from the body into various concentrations of lactic acid in normal saline. The effect of different nutritional states of the animals on the type of change was also studied in this group.

Group 3. Bacterial and Parasitic Injury: Injection of bacterial filtrates, living cultures or parasitic substance intravenously, intraperitoneally or intramuscularly.

Group 4. Pharmacological Injury: Injections of strychnine and insulin in fasted and normally fed animals.

Amytal by intraperitoneal injection was used to obtain complete anesthesia for all operative purposes. Injections into blood vessels were made with hypodermic needles so that the blood flow was disturbed as little as possible during the injection. Tissues were stained with Harris' hematoxylin and eosin and with Mallory's triple connective tissue stain.

Type protocols are given below.

Group 1. Physical Trauma

1. Ligation Alone: Femoral artery of one side of amytalized rabbit ligated with aseptic technique. Animal killed by blow on back of head 18 hours later. Gastrocnemii removed at once and placed in 10 per cent formalin at 37.5° C.

Control muscles showed no abnormal change grossly or microscopically. Ischemic muscles showed grossly merely marked pallor. Microscopically there is slight change. Some fibers are moderately swollen, stain feebly with eosin and show scattered fine granules in the cytoplasm.

2. *Contusion:* (*a. With Ligation:*) Femoral artery on one side tied off. Gastrocnemius on that side injured by sharp blows with a wooden board, and hamstring muscles on same side injured by pinching with heavy forceps. Animal killed by sharp blow on back of head from 2 to 5 hours after ligation.

Both muscles showed grossly pallor, swelling and mottling with pinkish red and grayish white streaks. The tissues were very friable and opaque. Histologically there is considerable change, from swelling of the fibers with loss of cross striations to complete lumpy disruption of the contractile substance. A few fibers show vacuolization of the sarcoplasm. In many of the extremely degenerated fibers there are breaks in the sarcolemma sheath. Normal and completely disrupted fibers may lie side by side. No hemorrhage is evident and the vessels appear to be drained of their blood. In the gastrocnemius of one rabbit, in which both the femoral artery and vein were ligated, extensive vacuolization of the fiber substance was observed.

(*b. With Circulation Intact:*) Gastrocnemius and hamstring muscles of the other leg, with circulation intact, injured as above, and lumbar muscle bruised with wooden board.

The muscles were dark red in color from hemorrhage which stained the ruptured fibers and were tensely swollen and easily torn with handling. Microscopic examination reveals marked and extensive damage. Muscle bundles are broken up and the fibers show considerable disruption. There are two main types of change: (1) Some of the fibers are smooth and hyaline, staining darker red than normally with eosin, and their cross striations are either indistinct or absent. In some of these the cytoplasm is clouded with

fine granules. The sarcoplasm of others seems to be alternately condensed and rarefied into discoid segments without, however, any intervening breaks in the continuity of the contractile substance. (2) More of the fibers are swollen, with loss of cross striations and prominence of longitudinal fibrils, which tend to separate from one another with a vacuolization, which in extreme cases gives the fiber the appearance of a network. In most of the fibers the sarcolemma is intact, but occasional ruptures are seen. Numerous erythrocytes are seen in the interstitial tissue.

3. Hyperpyrexia: Examinations were made of the recti abdomini and diaphragmatic muscles of two dogs whose body temperature had been raised to 110° F for one-half hour by means of a high-frequency machine. The dogs became ill and died in a few hours after the treatment.*

No abnormal changes of the muscles were noted grossly or microscopically.

4. Freezing: The gastrocnemii of several rabbits were frozen quickly *in situ* with a slush of carbon dioxide snow and ethyl chloride. 4 to 72 hours later the muscles were removed and placed in warm formalin.

Grossly they were moderately swollen, and surrounded by a pink to yellow fibrin exudate. Microscopically there is moderate patchy edema. The muscle fibers show all grades of degenerative change from simple swelling to complete disruption, as in those injured by mechanical trauma, although less extensive and usually showing fewer of the severely injured fibers. There is marked hyperemia but no thrombosis of the vascular channels. Exudative cells are rather few, and the majority are mononuclear phagocytes with a smaller number of polymorphonuclear leucocytes.

Group 2. Chemical Trauma

1. In Vitro Experiment: Animal killed by sharp blow on back of head. Large portions of lumbar muscle were excised and cut rapidly into blocks about 5 mm. in thickness, which were immediately dropped into lactic acid solutions of various concentrations in saline at 37° C for varying lengths of time. The lactic acid concentrations varied from 0.001 per cent (0.00011N) to 1.0 per cent (0.11N),

* We desire to thank Dr. Bernard Mortimer for the opportunity of studying the muscles of these animals.

and the time intervals from 30 seconds to 1 hour. Each of the forty-five specimens thus obtained was stirred in a large volume of warm 10 per cent formalin immediately on removal from the acid.

No gross muscle change was discernible. Microscopically the muscles are without change except at the ends of the fibers, which are swollen and knob-like and show loss of cross and longitudinal striations. Even in the weakest acid solutions, as well as in the strongest, the same changes are seen.

2. *In Vivo Experiments:* (*a*) Warm lactic acid (2 per cent in saline) injected into femoral artery over a period of from 3 minutes to 1 hour in normally fed animals, into animals fasted 4 days, and into one animal in which a lipemia had been produced by the feeding of 130 cc. of cream by stomach tube 4 hours before the beginning of the injections. All animals were sacrificed immediately after, or within 25 minutes of cessation of injection, vessels ligated, and the muscles removed at once and placed in warm formalin.

Gastrocnemii injected with 5 cc. of 2 per cent lactic acid over a period of 3 to 5 minutes showed no change grossly or microscopically.

Gastrocnemii injected with from 25 to 50 cc. of 2 per cent lactic acid over periods ranging from 5 minutes to 1 hour showed occasional, irregular, zig-zag streaks of grayish white crosswise in the somewhat edematous muscle. The tissue was pallid and translucent, but not more friable than normal. Histologically various types of muscle degeneration are seen. Some fibers are swollen, opaque and have a shining hyaline appearance, staining more deeply with eosin than normal fibers. These fibers have intact nuclei and sarcolemma sheaths. Other swollen fibers are pale staining, with indistinct cross striations and conspicuous longitudinal fibrils. Their sarcoplasm tends to be alternately rarefied and condensed, although no actual spaces are seen. In some fibers there is exaggeration of cross striations, which look as though they were set in a colorless matrix. Occasionally there are small areas in which the fibers appear to have normal morphology.

(*b*) 135 cc. of warm 2 per cent acid injected into femoral vein over a period of 1 hour. Dyspnea noted during injection, especially during the times when the acid was actually flowing into the vein, and less marked during the short rest periods.

Muscles of the legs, back and diaphragm showed no abnormal change.

(*c*) Intramuscular injection of 2 cc. of warm 2 per cent acid into the lumbar muscles of a lipemic animal whose femoral artery had also been injected. Animal sacrificed $1\frac{1}{2}$ hours after lumbar injection, and immediately after the arterial injection.

Lumbar muscles showed at site of injection a slight hemorrhage with diffuse surrounding edema. The whole area measured about 4 cm. in diameter. The tissue was opaque, grayish white in color and friable. There is interstitial edema throughout the sections. Many fibers show swelling and loss of cross striations. Longitudinal fibrils are often prominent. The typical change is a vacuolization, small to large, with separation of fiber substance.

Leg muscles on injected side are similar to the above-described muscles of non-lipemic animals.

Group 3. Bacterial and Parasitic Trauma

Materials: A highly virulent strain of *B. mucosus capsulatus* was obtained at autopsy from the lung of a man who had died of pneumonia. The stock culture was kept on blood agar, transfers being made as needed to dextrose agar slants containing a small quantity of broth.

The strain of *Streptococcus hemolyticus* used was obtained from the lungs of a rabbit which had died of empyema.

The strain of *Streptococcus viridans* was obtained from the infected tooth of a man.

In all instances the cultures used for injection were 24 hour growths in nutrient broth.

The *Ascaris lumbricoides* were obtained from sheep intestines at the slaughter house. The worms were washed thoroughly in water, dried in a current of slightly warmed air, ground up finely and passed through a fine screen so that an impalpable powder was obtained. Injections were made of this powder suspended in physiological salt solution.

The diphtheria toxin used had an M. L. D. of 0.004 cc.

1. Intraperitoneal Injections Into Mice of 1 or 2 cc. of Living Cultures of Streptococcus hemolyticus and of B. mucosus capsulatus: All animals became extremely ill very shortly. The streptococcus-inoculated mice were killed with ether in about 10 hours, the others died within 4 hours. Muscles of the entire body were fixed at once in 10 per cent formalin.

Grossly there was marked, acute peritoneal inflammation with fibrinous or fibrinopurulent exudate, but no abnormal skeletal muscle changes were observed.

Microscopically there is little change. The diaphragmatic and heart muscles show the finely granular change characteristic of cloudy swelling. The only other change is seen in the fibers surrounding a small abscess in the abdominal wall of one animal at the point where the needle entered. The altered fibers in this zone are completely hyalinized and glistening and take a deep eosin stain.

2. *Intravenous Injections Into Rabbits of Living Cultures of Streptococcus hemolyticus and of Streptococcus viridans:* Rabbit X3 1 cc. *Streptococcus hemolyticus* into ear vein October 23, 1 cc. on October 30. Rectal temperature at time of first injection 104.5° F; 5 hours later 104.4°; 24 hours later 105.5°. Temperature 30 minutes after second injection 105.5° F. Rabbit died 1½ hours later. Tissues fixed at once in Zenker's fluid.

No gross changes were observed and microscopic examination shows only slight cloudy swelling, most marked in the heart.

Rabbit X4. 1 cc. of *Streptococcus viridans* into ear vein October 23, and 10 cc. October 30. Animal ill. 20 cc. intracardiac on November 7. Animal died at once. Muscles fixed in formalin and Zenker's fluid. Rectal temperature at time of first injection 103.2° F; 5 hours later 106.1°; 10 hours later 104.8°; 22 hours later 103.3°; 27 hours later 103.5°. Rectal temperature at time of second injection 104.8° F; 24 hours later 104.9° F.

Autopsy revealed hemopericardium and fibrinopurulent pericarditis with petechial subpericardial hemorrhages. No gross skeletal muscle changes were observed.

The lumbar muscles show no microscopic changes. The diaphragmatic and leg muscles show occasional areas in which the fibers are swollen within the sarcolemma sheath. Cross striations are indistinct or absent and fine granules may be seen throughout many of the fibers. Here and there are groups of fifteen to forty muscle nuclei gathered within one sarcolemma sheath. A few mononuclear phagocytic cells are present in the interstitium.

3. (*a*) *Intramuscular Injections Into Rabbits of Living Cultures of Streptococcus hemolyticus, Streptococcus viridans, and B. mucosus capsulatus:* Rectal temperatures showed no significant alteration. Animals sacrificed in 40 to 50 hours.

(*b*) *Ascaris Substance Injected Intramuscularly:* Animal killed 24 hours later.

All injected muscles fixed in formalin and Zenker's fluid.

At the injected site in the muscles was a central yellowish gray area which was moist and friable. This was surrounded by a narrow, pinkish red zone, outside of which the muscle appeared to be unchanged. Affected muscles showed occasional grayish white spots a few millimeters in diameter.

Microscopically the inoculated site is outlined by a zone of polymorphonuclear leucocytes, outside of which the fibers are separated by edema fluid. There is dimming of the cross striations and moderate swelling of the fibers. Some of them show beginning disruption of the cytoplasm into irregular masses.

In the center of the inoculated area there is muscle destruction and pus cell infiltration. Bordering this central necrotic area there is an irregular zone in which the muscle fibers show varying degrees of degeneration. Many fibers are swollen and most of them are hyaline with indistinct or absent cross and longitudinal striations. In many the longitudinal striations become more distinct and occasionally tend to pull apart slightly. Some take a pale eosin stain, while a few become plump, opaque, take a deep eosin stain and have a glistening appearance. In many of these fibers there is disruption of the cytoplasm into irregular masses which separate, leaving clear spaces within the sarcolemma sheath. There is partial absorption of these degenerated fibers.

An isolated mass which resembled lymphoid tissue was found in the adductor muscles of a hind leg of one rabbit. It was removed to warm formalin. The mass measured 3 by 2 by 2 cm. and was attached only along its border next to the femur. It was surrounded by a reddish yellow zone of exudate. On section the tissue was pinkish gray in color, soft and succulent, and very friable. Microscopic examination reveals an isolated muscle mass surrounded almost entirely by a zone of young granulation tissue. The interior of the mass is composed of degenerated muscle fibers. These are swollen, entirely homogeneous and glistening in appearance, and take a strong eosin stain. Some of these masses show a breaking down into granular clumps with disruption of the sarcolemma sheath. The fibers are in general rather widely separated and numerous pus cells and débris are present. At one end regeneration of

muscle is seen, with myriads of young muscle sprouts present. A regional lymph node shows marked diffuse lymphoid hyperplasia.

4. *Diphtheria Toxin Injections:* 2 cc. of undiluted toxin intramuscularly; 30 cc. of toxin diluted 1:3 with saline intra-arterially with circulation intact; and perfusion of the leg muscles with 1:3 toxin; all within 1 hour. Animals sacrificed at once and muscle fixed in formalin.

There is hemorrhage and edema in the directly injected muscles. The others show only pallor.

Microscopically the directly injected muscles show moderate edema of interstitial tissues with moderate swelling of fibers, indistinct cross striations and prominent longitudinal fibrils. Nuclei and sarcolemma sheaths are intact. The perfused muscles show no abnormal change.

Group 4. Pharmacological Trauma

1. *Strychnine, by Subcutaneous Injection: (a) 4 mg. per Kilo Into a Normally Fed Rabbit:* Gave hyperirritability, rigidity, prostration, with muscle twitchings and convulsions beginning in 3 minutes, and death 15 minutes after injection. Muscles of legs, back, neck and diaphragm removed at once and placed in formalin.

Grossly there was no abnormal change, but microscopic examination revealed some muscle damage. In the diaphragm occasional fibers show granular degeneration of the cytoplasm and loss of cross striations. Some of these show further degeneration into broken granular masses inside the sarcolemma sheaths. Many muscle nuclei are seen, often irregularly distributed throughout the granular mass and surrounded by small cytoplasmic rims. Occasionally these degenerated fibers are completely fragmented and in one place where a few such fibers are broken across there is a small effusion of blood mingled with muscle fiber fragments and numerous muscle cell nuclei.

Sections of the leg and back muscles show swelling of the fibers at their points of attachment to the fascia, where there is loss of cross striation with prominence of longitudinal fibrils and separation of the longitudinal fibrils from each other. Some fibers show a diffuse opacity, due to clouding with fine granules. The sarcolemma sheath is intact. The remainder of the fibers have normal morphology.

(*b*) *2 mg. per Kilo Injected Into a Rabbit Which Had Been Fasted for 4 Days:* Convulsions beginning 1 hour later and spastic paralysis of the hind legs. Second dose of 1 mg. per kilo 2 hours after the first resulted in a severe convulsion and death 20 minutes later.

Sections of muscles of back, legs, and the intercostal and abdominal muscles show no change.

2. *Insulin, 10 Units, Injected in Upper Back Muscles of a Rabbit Which Had Received No Food for 43 Hours:* 3 hours later, after growing restlessness and hyperirritability, a series of mild convulsions lasting for about 10 minutes. 8 more units of insulin injected 1 hour later into upper back muscles of other side. 2 hours later strong convulsions, legs spastic. Convulsions at intervals. Animal growing weaker and less responsive. Killed by blow on back of head 7 hours after first injection.

No significant alterations observed in muscles of the legs, diaphragm, abdomen or the intercostals.

Discussion

As might have been expected, the early gross evidences of muscular trauma caused by pinching and striking of the muscle with intact circulation are swelling and hemorrhage. The friability results partly from the traumatic separation of fibers, as well as from the degeneration which occurs. Microscopically this degeneration is found to comprise various types of fiber damage. The beginning change is simple swelling with loss of cross striations and fading of the eosin-staining property. In many of these fibers the longitudinal fibrils stand out distinctly. In such fibers the further progress of degeneration is marked most characteristically by vacuolization. When this change is extreme the swollen fiber appears to be made up of a filmy network of bubbles. According to Wagener [16] this vacuolar change is a purely degenerative process which results in complete destruction of the fiber. Other fibers, usually scattered singly here and there among intact fibers or among fibers showing other types of injury, are strikingly marked out by their bright red color, opaque appearance and completely hyaline structure. They have a shiny look. Rarely the swollen fibers have the appearance of cloudy swelling, with their cross striations partially or completely obscured by the appearance of small glistening granules which

sometimes have a slightly yellowish tinge. This granular appearance may, according to Nesti,[15] presage the final conversion of the fiber into the waxy form of degeneration.

In all of these described forms of degeneration the sarcolemma sheath may be intact, with the contained muscle nuclei uninjured. Many sheaths, however, have been ruptured from the force of the trauma, and in these the muscle nuclei may be found escaped in the outpoured cytoplasmic mass in varying stages of degeneration, or at times completely degenerated.

As to the underlying factors leading to muscle degeneration in this type of trauma, the actual traumatic agent must be considered first. It might be expected that trauma to the muscles would be more effective if their circulation were impaired, since this would facilitate accumulation of toxic metabolic products. In the muscles injured by striking and pinching after ligation of the arterial blood supply extensive degeneration of the fibers is evident. According to Thoma [17] this decreased resistance of fibers is due to lack of nourishment, the combination of decreased food and mechanical injury producing the degeneration which, according to him, either injury alone fails to effect. In our experiments, however, about as much damage was observed with intact circulation as in the ligated muscles. Also, since degeneration does occur without hemorrhage, the factor of blood effusion cannot be considered a basic factor in the causation of the degenerative change.

The damage produced by ligation alone is of slight extent and compares with the change seen in parenchymatous organs with cloudy swelling. This lack of significant change with simple ischemia is affirmed by Thoma [18] and Wells.[19] Contrary opinions, on the other hand, are expressed by Volkmann [2] and Siegmund.[20] Voelcker (cited by Krogius [21]) was the author of a theory that the basic cause of congenital torticollis is pressure of the shoulder of the infant *in utero* upon the upper end of the sternocleidomastoid muscle where the artery enters, with resultant ischemic degeneration of that muscle.

The effect of increased body temperature on the incidence of muscle degeneration has been considered by Fahraeus (cited by Stenström [6]), and Ghedini and Fedeli (cited by Wells [7]), who are agreed that the muscle is unaffected by fever. In our dogs with an extremely high body temperature the muscles studied showed no

gross or microscopic evidence of injury. The rabbits which were checked for body temperature following bacterial injections showed no connection between temperature observed and muscle degeneration resulting. As to the effect of temperatures considerably above those developed by the animal body, Volkmann [2] made observations on muscle degeneration caused by burning, cauterizing, and injecting hot water, and found not waxy degeneration but complete necrosis of the affected muscle, with healing by scar formation.

In addition to the actual physical damage of the traumatized muscle there is set up in the injured area an alteration of metabolism which may lead to accumulation of damaging products. It is logical to assume that one product accumulating rapidly in such a situation is lactic acid. In 1909 Wells [19] first advanced the theory that waxy degeneration of skeletal muscles is caused by this collection of lactic acid in the muscle. He found a striking homogeneity of the swollen fibers resembling that of Zenker's degeneration, when he placed muscle tissue into solutions of lactic acid in saline. Even as dilute a solution as 1:64N lactic acid gave, in an hour or two at 37° C, distinct swelling of the ends of the fibers, loss of transverse and obscuration of longitudinal striations. Since sodium lactate did not produce this change and hydrochloric acid did, Wells concluded that it was the hydrogen ion which was the important factor. Wells gives the figures of Fletcher and Hopkins [22] which show that enough lactic acid to produce the change can accumulate in living muscle. Although their methods have since been shown to yield too high figures, more recent workers [23] have obtained even higher values for rat muscles exercised to complete fatigue.

In our experiments with lactic acid *in vitro* no muscle change was observed. The cut ends of the fibers were knob-like and hyalinized in specimens at all the lactic acid concentrations studied, but since similar changes were observed in the controls which were placed in saline solution, this change was interpreted as being due to rupture of the fiber sheath in cutting the sections, as reported by Thoma in 1909.

Quite different results are seen in the living animal injected with lactic acid, either into the blood supply of a part of the body or directly into the muscle. The most extensive changes are noted on the direct injection of the injurious agent into the muscle. No detectable differences are observed in muscles of the animal with

an artificial lipemia. The lipemia was created before the injection of the acid to determine whether a difference in the amount of available fat in the circulation might influence the extent of muscle degeneration, since Rumpf and Schumm (cited by Wells [24]) found an increase of fat amounting to about fifteen times the normal amount, in muscles showing "reaction of degeneration."

As with lactic acid, so with bacterial cultures, direct injection into the muscle causes marked degeneration. It is indicated that the effect is obtained largely by the direct action of the bacteria or their products upon the muscle fibers, for when much larger amounts of the same cultures are injected intravenously little or no degenerative muscle change is evident. The same negative results are found with acute infection of the peritoneum by virulent organisms. Since the peritoneum offers a relatively huge surface from which absorption is rapid, and since septicemia is a probable accompaniment of the peritonitis, toxins must have been present in the circulation in large amounts in these cases without, however, causing serious muscle damage.

Our findings, therefore, confirm the opinion of Beneke and Steinschneider [11] that the muscle damage is produced by direct injury to the contractile substance. These workers investigated the effect of direct bacterial injury to the muscle. Thoma, however, ascribed to bacteria an indirect rôle — that of disturbing the nutrition of the muscle and so rendering it susceptible to physical trauma. MacCallum [25] believed that the living bacteria which he found in degenerated muscle areas were secondary invaders after primary toxic injury.

Diphtheria toxin injected in large amounts, with or without active circulation, gave no change. Direct injection of toxin into the muscle produced only interstitial edema, which may be attributed to the volume of fluid injected.

Anaphylaxis has been suggested as a cause of waxy degeneration of muscle by Beneke and Steinschneider in 1912, and by Wells in the same year. In one of our rabbits (X4) anaphylaxis must be considered the probable cause of death, and in this animal slight degenerative changes were found in the muscles of the legs and diaphragm.

It is probable that in local infections of muscle the disturbances of circulation from stasis and from thrombosis of blood vessels is

added to the injurious effect of the bacteria. The extent of the muscle injury which can result from such a combination of factors is shown in one rabbit, in an isolated muscle mass resulting from a spontaneous infection. Here the muscle degeneration was extreme. A similar type of muscle mass was found by Loeper and Lemaire [26] in the cervical region of a man.

Closely related to the type of injury produced by direct bacterial injections is that resulting from injection of ascaris substance into the muscle. The injury with this agent may have resulted directly from the parasitic toxins.[27, 28]

Sudden violent contraction of muscle was shown to give varying results as regards degenerative changes. In the normally fed rabbit administration of strychnine resulted in definite granular degeneration of muscle fibers and even rupture, with slight hemorrhage. In contradistinction, in fasting animals injected with strychnine and with insulin, although the convulsions were just as violent the muscles appeared unchanged. Zenker had already noted that waxy degeneration often did not occur in those muscles most involved in tetanic contractions, while appearing extensively in other muscle groups. Our findings corroborate this observation and support the view that violent muscle contraction *per se* does not constantly produce this change. Borst [3] believed that it was rupture of fibers from forced contractions that led to their degeneration, while according to Stemmler [13] rupture occurs only in those fibers which have been previously injured, as, in his experiments, by tetanus toxin.

Conclusions

The types of muscle injury resulting from different injurious agents used varied from the slightest clouding of the cytoplasm to complete necrosis of the fiber substance. The earliest change is that which was described as being similar to cloudy swelling in parenchymatous organs. By this analogy we may presume further that such fibers might recover from their damaged state without either regenerative or other reparative process. A favorable ending might be anticipated also for the milder forms of true granular degeneration (Virchow [29] and Zenker [1]) whether this is of the albuminous or fatty type. With greater fatty change, however, according to Zenker, the cell is irreparably damaged. The albuminous and

simple hyaline forms of degeneration progress further, according to Nesti,[15] into the waxy form. This waxy form is generally agreed to be completely homogeneous and of altered protein composition. The presence of fat in this waxy material has not been definitely established although, according to Forbus,[30] fat sometimes appears in the vacuoles resulting from the dissolution of waxy material.

Further degeneration of the waxy fiber is marked by its disruption into irregular masses separated by clear spaces (Zenker's "Schollige Zerklüftung") or by its breaking up into coarse granular particles.

Another form of degenerative change is seen in the edema of the muscle fiber accompanied by dimming of cross striations and marked prominence of longitudinal fibrils, which results from mechanical and chemical trauma. This degenerates further with the formation of vacuoles which increase in size until they occupy practically the whole space of the fiber, giving it the appearance of a network.

Muscle nuclei are present in most of the fibers which are injured by mechanical trauma, but tend in general to be absent in those fibers injured by chemical and bacterial means.

Severe mechanical trauma frequently causes rupture of the sarcolemma, although marked degeneration is frequently seen in fibers with intact sarcolemma sheath. In other types of injury the sarcolemma sheath generally remains intact until extensive destruction of fiber occurs, although it is often unbroken, even with almost complete disappearance of the contained protoplasm.

In all the types of trauma studied, fibers with normal staining reactions and with intact striations are seen lying next to fibers which show varying degrees of degeneration.

It seems questionable that the noxa alone is the direct cause of the degenerative muscle changes. Since so many widely varied agents may produce similar pictures, it is likely that the trauma sets in action some principle already present or called into being in the muscle itself. This may be the altered reaction of the muscle, due to lactic acid accumulation, although the highest value of lactic acid found in a series of injured muscles was only 103 mg. per cent.[31] This is no higher than may be found in muscle fatigue [23] from which recovery is prompt, or within six minutes postmortem,[32] which is less time than elapses before the average normal muscle specimen is fixed for histological examination. It is likely that some other

factor, perhaps of enzyme nature, is active in the degenerative transformation of muscle fibers which are already injured by some non-specific agent.

It is evident from the study of these experimental muscle lesions that there is a series of degenerative changes as described above. The stage of hyaline, or so-called waxy, degeneration is but one phase of a progressive process. There is no more reason to use the name descriptive of one stage than of any other stage in naming the entire process.

Since the degeneration is often widespread and affects the contractile units of the muscle, we would suggest the name "acute molecular degeneration of striated muscle" as being more definitive and descriptive of the entire process.

SUMMARY

1. Degeneration of skeletal muscle has been produced by different types of trauma.

2. The types of trauma used were physical, chemical, bacterial and parasitic, and pharmacological.

3. The stages of muscle degeneration produced were:

(*a*) Slight granular clouding with swelling, and dimming of cross striations.

(*b*) Edema of fibers with prominent longitudinal fibrils.

(*c*) Vacuolization.

(*d*) True granular degeneration (*a*) albuminous or (*b*) fatty.

(*e*) Waxy degeneration, with further (*a*) lumpy disruption, or (*b*) granular disruption.

4. The name "acute molecular degeneration of striated muscle" is suggested as a better descriptive and more inclusive term than "waxy degeneration."

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DESCRIPTION OF PLATE

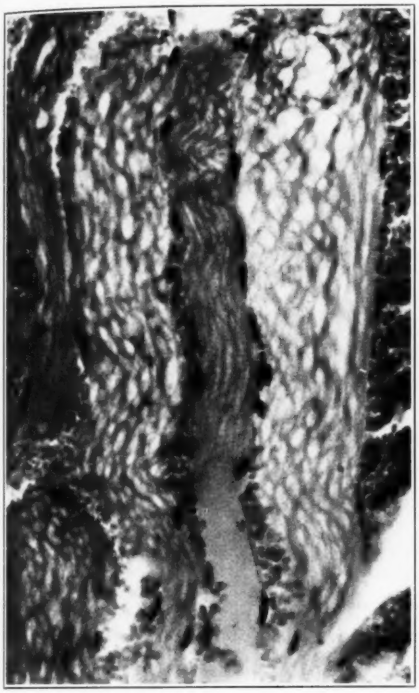
PLATE 34

FIG. 1. Vacuolar change of muscle fibers. × 325.

FIG. 2. Waxy change. × 325.

FIG. 3. Lumpy disruption of degenerated fibers. × 150.

FIG. 4. Profoundly traumatized area of muscle, with marked edema and ruptured fibers showing various types of degenerative change. × 150.



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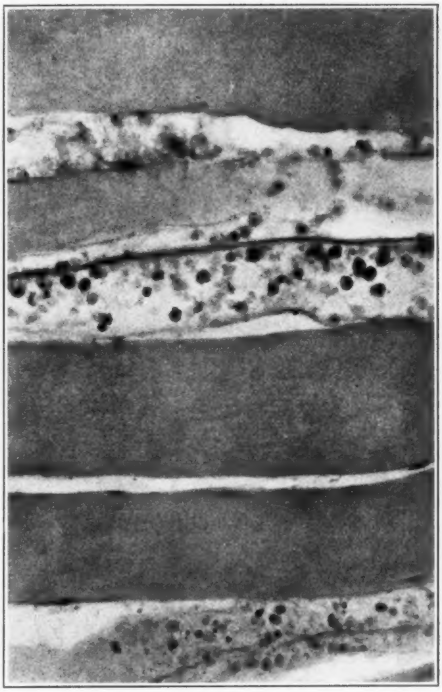

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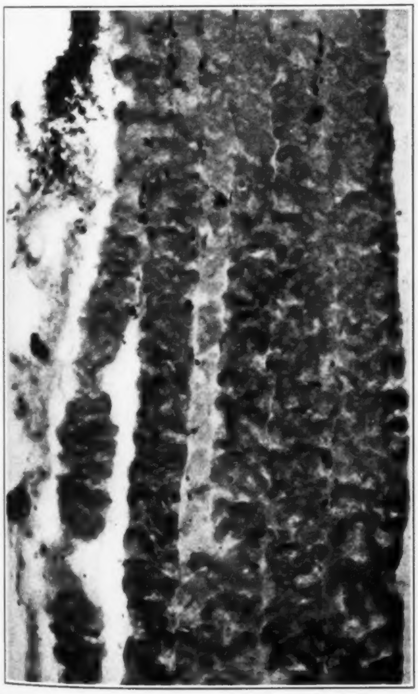

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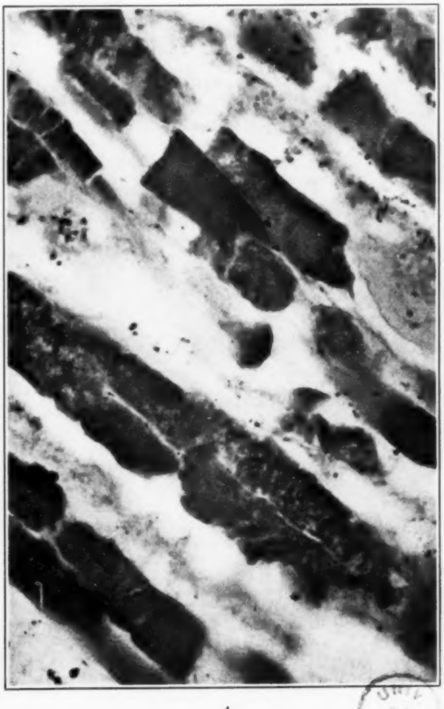

4

Fishback and Fishback Studies of Experimental Muscle Degeneration. I

STUDIES OF EXPERIMENTAL MUSCLE DEGENERATION *

II. STANDARD METHOD OF CAUSATION OF DEGENERATION, AND REPAIR OF THE INJURED MUSCLE

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In a previous paper [1] we have called attention to the experimental production of striated muscle degeneration in which hyaline changes of the muscle fibers constitute one stage of a progressive degenerative process. We have suggested the name "acute molecular degeneration" for this form of muscle change. This change was shown to be produced by a wide variety of traumatic agents. There is considerable difference among such agents, however, as to the extent and severity of the muscle lesions. They differ especially in ease of control of the experimental lesions produced.

After many trials with various methods one was finally found which met our requirements. The method of injury consisted in contusing the muscle while the animal (rabbit) was under light ether anesthesia. With the leg extended on a well padded wooden block the gastrocnemius muscle was struck a number of scattered light blows with a light, rubber-covered iron rod. After examining several muscles injured by this means, the operator could estimate fairly accurately at the time of injury the grade of muscle damage caused.

The use of this method was of advantage for several reasons. As stated, the amount of damage to the muscle was controllable. The animal was left in good general condition, so that the injured muscle might be studied at any stage of its progress. The skin was unbroken, with danger of infection thus minimized. No toxic or chemical agent was introduced which might interfere with subsequent chemical study of metabolic processes in the injured muscle.

With this standard method of causing a characteristic and reproducible degeneration of muscle a series of chemical studies was undertaken in the acute stage and during the progress of repair.[2]

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It also seemed worth while to report morphological studies of the injured muscles during the repair so that a more complete picture might be had of this standard muscle injury.

Gross Appearance of Injured Muscle

At 4 hours after injury the muscle is swollen to about double its ordinary size. There is edema of the subcutaneous tissues and of the injured muscles. The color is slightly cyanotic, as from blood stasis. Here and there are small, irregular, transverse, opaque, grayish white bars dotted in the muscle tissue. The muscle substance is somewhat more friable than normal.

Within 1 to 3 days the muscle loses its bluish red color and has a distinct salmon color, with contained areas of opaque grayish white tint. These areas are quite large, usually making up the greater part of the muscle, are irregular in shape and shade off at the margins into the more normal appearing muscle structure. The cut surface is not moist and glistening as in normal muscle, but appears dull or opaque and somewhat dry. The tissue is firm and quite friable.

The 5 to 10 day periods show an increasing tendency to easy bleeding of minute vessels in the area immediately surrounding the muscle, associated with apparent organization of a fibrinous exudate between the muscle and its fascia. The muscle size is decreased somewhat from the early stage of acute swelling. On section at 10 days there are small scattered areas of glistening moist tissue with the pinkish red color of normal muscle. The degenerated areas of opaque, grayish white, friable tissue show at this time a narrow, bright red to yellow-red marginal zone.

With longer periods of repair the muscle gradually diminishes in size but is still larger and more tense than normal at 28 days. The exudate between the muscle and fascia becomes firmly organized by 16 days. The proportion of more normal appearing muscle becomes much greater with disappearance of the degenerated areas, which have not entirely disappeared, however, by the end of the observation period. At their margins there is an advancing zone which fades from red to reddish gray in the late period. This zone is somewhat translucent and resists tearing. The amount of such new-forming connective tissue is never large proportionately, even in the latest period.

Microscopic Appearance of Injured Muscle

Within 4 hours after injury there is considerable interstitial edema with some fibrin in the edema fluid. The blood vessels show moderate dilatation, but no thrombosis is evident. A few extravasated erythrocytes are found here and there, but remarkably few for the severity of the injury. In earlier experiments, in which an unpadded wooden board was used in the production of the injury, considerable hemorrhage was occasionally found. The muscle fibers are extensively damaged. Many are ruptured transversely, some completely through the sarcolemma sheath, others only through the myofibrils, with intact sheath. Complete rupture of the fiber results in a clubbed appearance of the fragments, due to retraction of the sarcolemma and extrusion of sarcoplasm. This knobbed end is of smooth hyaline form. Muscle nuclei in these fibers are usually retracted into the fiber and grouped together.

Various early degenerative changes are found. Some few fibers have lost their cross striations, which are, however, retained in other fibers. Longitudinal fibrils are emphasized and a few small vacuoles are seen. The nuclei of the muscle fibers and sarcolemma sheaths appear to be unchanged. The staining reaction of the tissue shows but little alteration, the fibers with swelling and lost cross striations staining somewhat paler than normal.

Separation of fibers by edema brings into prominence occasional scattered muscle fiber sprouts in some of the sections. These are very small new fibers with pointed tips, many nuclei, and no cross striations. Nauwerck (cited by Craciun[3]) found marked nuclear division beginning 4 to 6 hours after experimental muscle injury, and interpreted it as the first evidence of repair of that injury. In the present study we interpret the nuclear proliferation seen at this very early stage as evidence of repair, not of the damage due to the experimental procedure, but of muscle fiber degeneration sustained during the ordinary activities of the animal. There are very few such proliferating fibers seen in all the sections studied, so that the nuclear growth is not marked enough or widespread enough to be due to the experimental damage to the muscle. Other workers have also considered similar nuclear groupings as part of a non-pathological process, since they were seen in normal animals. Heiden-

hain, Korotneff, Stemmler, Morpurgo, and Schütz (cited by Craciun[3]) associated them with physiological repair.

Within 24 hours after injury there is added to the previous picture a moderate diffuse infiltration by polymorphonuclear leucocytes and lymphocytes. The muscle fibers show extensive loss of cross striations and hyalinization of the cytoplasm. There is considerable proliferation of sarcolemma nuclei at the points where the sheath is ruptured, and some very early growth of muscle cells. In some places where there is marked damage to the sheath these proliferating muscle cells are turned directly at right angles to the fiber axis. Thoma[4] found muscle fiber proliferation in mechanically injured frog tongue muscle within 60 hours after injury.

At 48 to 72 hours after injury there is evident an increasing degree of damage. There is marked hyalinization of muscle fibers with disappearance of most of the muscle nuclei. Some hyaline fibers are already breaking up into lumpy masses or into disc-like segments. Accompanying the diminishing edema there are fewer fibers showing wide separation of longitudinal fibrils with vacuolization. Leucocytes at 72 hours are very few, and phagocytic cells containing débris are beginning to appear. Occasional normal fibers are seen lying next to badly degenerated fibers at all stages of the muscle injury.

It appears strange that evidence of regeneration should be less conspicuous at 72 hours than at 24 hours after injury. Study of the earlier sections, however, leads to the conclusion that proliferation is started by the muscle nuclei set free by the breaking up of severely traumatized fibers. This proliferation seems to proceed no farther than the sending out of delicate fibrils by individual cells, which are then involved in the progressively deepening muscle degeneration. It seems likely that this first attempt at repair, then, is only a primary response to trauma by nuclei which are too badly damaged to carry on.

At the end of 5 days there are still many large islands of hyalinized fibers within which there is practically no cell infiltration or proliferation. In addition to segmentation and clumping of the cytoplasm in these fibers there now appear in some of them irregular spaces with frayed margins, giving them a moth-eaten appearance. Marked early muscle cell proliferation occurs around the margins of such patches of fiber degeneration. There are numerous

irregular single cells with fibrillar extensions, and syncytial sprouts with multiple nuclei. Some of these latter appear within old sarcolemma sheaths. Fibroplastic proliferation is likewise beginning. Numerous phagocytes are present, some invading the degenerated fibers where they are at times arranged around the inside of the sarcolemma sheath, entirely surrounding a central core of degenerated cytoplasm. Such cytoplasm tends to show basophilic staining. There are a moderate number of lymphocytes in the interstitium. The various types of cells appearing in muscle regeneration were studied by Forbus [5] with the use of vital staining, which aided in proving origin but apparently did not by itself identify the cells better than morphological characteristics.

After 8 to 10 days the types of cells present are unchanged. There is considerable more breaking up of the hyaline fibers, with granular degeneration of the lumpy masses. In the areas of regeneration there is considerable basophilic staining of the degenerated fibers. Reparative effort is prominent, with clumps of new-growing muscle fibers up to 1 mm. or more in length. In the larger of these, longitudinal fibrils appear. Sarcolemma nuclei show proliferation. Occasional empty sheaths are found which, when partially collapsed, show striking resemblance to small capillary buds. A very few capillary buds are present, in contrast to the rich number seen in ordinary granulation tissue.

The 12 day period shows larger masses of more mature muscle fibers, in which definite cross striation appears. There is a gradual diminution in size of the areas of degenerated fibers in this and in each of the succeeding periods. At 16 days a cross-section demonstrates the new fibers in an area of repair, arranged in bundles with endomysial bands between them, forming a very faithful reproduction of the normal muscle picture.

In the 18 and 21 day periods a few areas of almost normal sized new fibers are found. An occasional young fiber is seen with all the nuclei of both the muscle and sarcolemma arranged at the borders and projecting like beads stuck on the surface of the fiber. It seems most likely that this is a fixation phenomenon.

Even at 25 to 28 days small numbers of hyaline degenerating fibers are seen. Scattered areas of newly formed, loose, fibroblastic structure are present in which are seen a few lymphocytes, phagocytes and single muscle cells. There are also isolated muscle fibers

with many nuclei, some of the fibers showing early degenerative changes. The majority of persistent new fibers are formed in groups, and these are larger and more normal in appearance than those seen during the earlier periods of study.

Summary

1. In acute molecular degeneration of striated muscle in rabbits the development of muscle fiber degeneration is a progressive process including edema, fibrillar separation, vacuolization, hyaline change, lumpy disruption, granular change and finally, complete dissolution of the muscle cytoplasm. This is associated with some cell exudation, with a high degree of phagocytic activity, and finally with repair.

2. The course of repair is toward regeneration. The completeness of this process appears to depend upon the destructiveness of the lesion, and not upon the extent or severity of muscle fiber degeneration. If the sarcolemma destruction is not too severe and the stroma remains, these, with surviving muscle nuclei, form the integral factors for muscle restoration. With diffuse tissue destruction scarring results.

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DESCRIPTION OF PLATES

PLATE 35

FIG. 1. 4 hours after trauma. Interstitial edema. Rupture of some fibers. Swelling, loss of cross striation and appearance of longitudunal fibrils in many of the fibers. × 170.

FIG. 2. 48 hour stage. Waxy change of fibers. × 325.

FIG. 3. 5 day stage. Proliferation of fibroblasts, sarcolemma and muscle cells. Early attack of phagocytes on degenerated muscle fibers. × 190.

FIG. 4. 8 day stage. Lumpy disruption and phagocytosis of degenerated fibers. Growth of young muscle sprouts. × 190.

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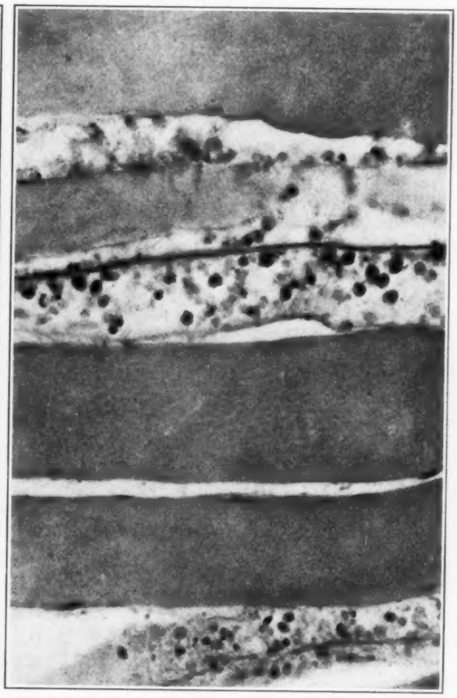

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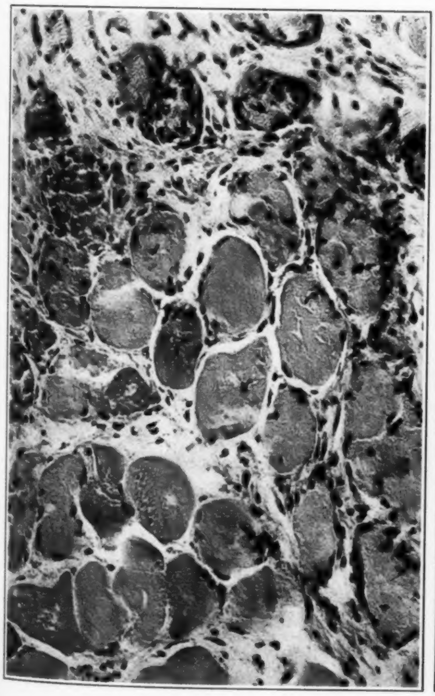

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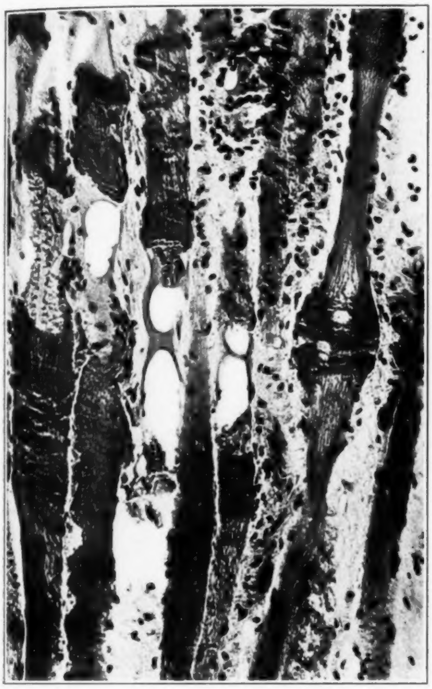
Fishback and Fishback Studies of Experimental Muscle Degeneration. II

PLATE 36

FIG. 5. 10 day stage. New-growing muscle cells connecting old degenerated masses within the sarcolemma sheath. Spindle forms and vacuolated fibers. × 190.

FIG. 6. 12 day stage. New-growing fibers with cross striations appearing. × 600.

FIG. 7. 28 day stage. Regenerated area with fairly normal muscle fiber arrangement. Some excess of interstitium. × 37½.



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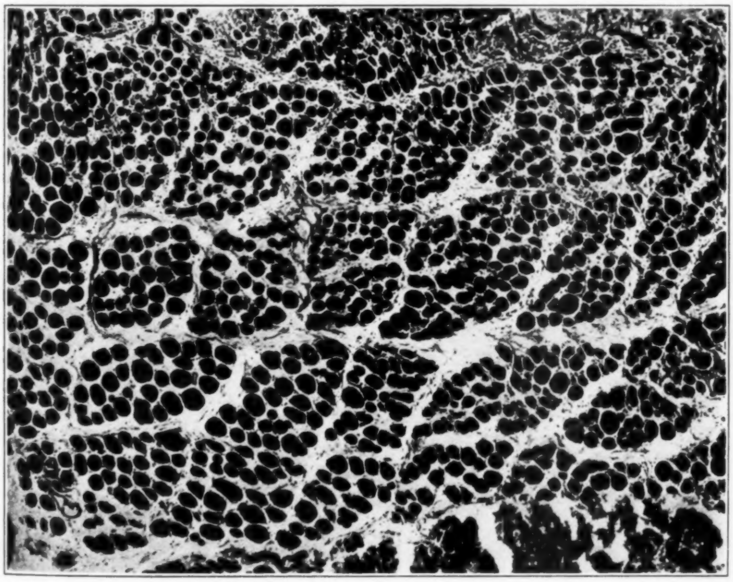

7

VITAL STAINING OF THE RABBIT'S AORTA IN THE STUDY OF ARTERIOSCLEROSIS *

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With the development of the imbibition or infiltration theory of arteriosclerosis (Ribbert,[1] Aschoff,[2, 3] Anitschkow[4, 5]), a new significance has been attached by some investigators to the behaviour of colloidal dyes introduced into the circulation. The relative ease with which the movements of dyes can be traced in the animal body offers particular advantages in the study of colloidal materials in the blood plasma, the properties of which are of special importance in relation to the imbibition theory.

The imbibition theory of "atherosclerosis" depends upon the assumption that fluids may penetrate the intimal surface of the arteries from the lumen. The existence of such an inflow, at least of true solutions, is generally admitted and is looked upon as the normal mode of nutrition of the intima and inner layers of the media. The theory further requires that lipoid materials in colloidal state, having penetrated the intima, may be deposited there under certain conditions, chief among which are a peculiar swelling and loosening of the intima and a sufficient concentration of lipoids, especially of cholesterin esters, in the plasma (Aschoff). The fatty substances are spoken of as being "pressed" into the intima. The experimental production of fatty deposits in the arteries of rabbits fed on high lipoid diets for long periods has been adduced as evidence in favour of the theory. However, in the aortas of both humans and experimental animals, the fatty changes are localized to certain distinct areas which are more or less characteristic for each. Various causes have been assigned for the localization of these deposits and recently the problem has been attacked through experimental studies on the penetration of various colloidal dyes into the walls of arteries. It was hoped that the behaviour of such dyes introduced into the

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circulation might furnish an analogy to that of colloidal substances normally present in the blood plasma. Moreover, such studies might demonstrate local variations in the permeability of the intima of the arteries to account for the localization of fatty changes in accordance with the imbibition theory.

The first systematic investigation into the ability of colloidal dyes to penetrate the walls of arteries was made by Petroff [6] in 1922. He was able to demonstrate staining of the walls of both veins and arteries in the frog's mesentery after intravenous or subcutaneous injection of trypan blue or lithium carmine solutions. The staining, however, was always diffuse. In rats and rabbits, after intravenous or subcutaneous injection of either of these dyes, he observed similar staining of the fine mesenteric arteries and veins. In their aortas he found, in short experiments, marked staining of the elastic lamellae in the inner and outer layers of the media, while the elastic fibres in the intermediate portion remained unstained. In longer experiments, in rats, the staining in the outer layers of the media was always somewhat deeper than that in the inner part. He also observed that application of sodium chloride crystals or weak solutions of hydrochloric acid or silver nitrate to the arteries brought about much deeper and more rapid staining of their treated portions after intravenous injection of trypan blue solution.

Okuneff,[7] a few years later, carried out experiments on dogs, cats, rabbits, guinea pigs, white rats and white mice, using solutions of trypan blue which were introduced by various routes (intravenously, subcutaneously, intraperitoneally) into the stomach and into intestinal loops. The last two methods of injection were not successful in most of the animals, in that the stain was not absorbed in sufficient quantities to produce visible staining of the aorta. The results from the other three methods of introduction of the dye were very similar to one another but varied considerably in the various animals used. In rabbits, guinea pigs, white rats and white mice, the staining of the aorta was always diffuse. However, in two rabbits and three guinea pigs, small blue patches of rounded outline were observed in the arch of the aorta and usually in its first part. A great similarity existed between the staining of the aortas of dogs and cats. This staining was described as being always fleck-like, although these flecks often coalesced to form larger patches of deep staining. The author called particular attention to the similarity between the distribution of

these deep staining areas in dogs and cats and that of the fatty deposits in the aortas of rabbits and in humans. After speaking of this similarity and the probability that common factors were responsible for the typical localization in both cases, he stated: "Nun erweist es sich aus meinen Versuchen, dass auch für eine andere Substanz kolloidaler Natur die gleiche Art des Eindringens in die Aortenwand anzunehmen ist. Die typischen Stellen der Lipoidablagerung in der Aortenwand sind in diesem Fall als Stellen zu betrachten, wo der Durchtränkungsstrom der Blutlymphe am stärksten ausgeprägt ist."

In experiments upon rats, guinea pigs and rabbits, Glasunow[8] used a somewhat different method. The animal was anaesthetized, the thoracic and abdominal cavities were rapidly opened and a cannula inserted through the left ventricle into the ascending aorta: another cannula was fixed in the vessel just above its bifurcation. The aorta was then perfused with a warmed and oxygenated solution of trypan blue in Ringer-Locke solution for periods varying from five minutes to one hour. In the thoracic aorta a fleck-like distribution of the dye was found. The flecks in the longer experiments coalesced to form dark staining patches, but the colouring was always deepest immediately beneath the mouths of the intercostal arteries, forming crescentic patches which were later joined by longitudinal lines on either side. In the abdominal aorta the staining was more diffuse, but a similar picture was seen about the mouths of the branching vessels. It was found also that short cauterization of the external wall of the aorta or crushing with a clamp resulted in deeper staining in the injured area. Glasunow concluded that the deeply staining areas which he noted corresponded to areas in which the permeability of the intimal surface was greater than elsewhere. It should be pointed out, however, that his method of investigation is open to certain objections. Not only might the normal permeability of the lining membrane of the aorta have been altered under the artificial conditions of the experiments, but also the properties of the dye in association with a simple saline solution might have been very different from those which it would possess in such a complex colloidal system as the blood plasma.

Quite recently, Hackel[9] has carried out experiments upon rabbits injected intravenously with trypan blue solution. His experiments lasted from thirteen to fifty minutes and during this time he produced an intermittent rise in blood pressure by clamping the innom-

inate artery for short periods, separated by intervals in which the clamp was released. In some of the experiments injections of adrenalin and electrical stimulation of the central end of the cut sciatic nerve were also used to increase the blood pressure. He found that the aortas were more deeply stained in those animals in which the blood pressure had been raised, than in control animals in which the same dissection had been carried out but in which the innominate artery had not been clamped. He concluded that an increase of blood pressure could produce a much more pronounced imbibition by the intimal surface of the aorta, not only of dye but also of other colloidal constituents of the plasma.

It appeared to the author that the broad conclusions drawn from the experiments quoted above were in many respects poorly grounded in the experimental evidence. It was felt that further investigation into the question might serve to clarify the situation and possibly provide an adequate explanation for the phenomena observed in vital staining of the arteries. With this object in view, the following experiments were undertaken.

Experiments

In this series of experiments only intravenous injection of dye was employed. It was felt that the method of Glasunow was too artificial to be entirely free from objection, while in the experiments of Okuneff no advantage appeared to be gained by intraperitoneal or subcutaneous injection and the dye was not absorbed at all through the normal mucosa of stomach or intestine. Rabbits were used exclusively since, in their arteries, the experimental production of localized fatty deposits is easily accomplished and therefore possible peculiarities in the lining membrane of their aortas should be easily demonstrated. Accordingly, rabbits were injected intravenously with varying quantities of a 1 per cent solution of trypan blue in Ringer-Locke solution, each animal receiving a single injection. In one case a 0.1 per cent solution of the dye was used. The animals were killed at the end of from one hour to one hundred and one hours. The aortas were immediately removed and opened, and the character of the staining in their walls and in other tissues recorded at once. Where the staining of the aorta was irregular the distribution and intensity of the colouring as seen on the intimal surface was recorded

as accurately as possible on "outline charts" of the aorta on which the staining was reproduced graphically with blue crayon. These records were thus available for subsequent comparison.

The following table gives the detail of the experiments.

TABLE I

Rabbit No.	Weight	Dose of trypan blue		Dose per kilo		Duration of experiment	Staining of the aorta as seen on the intimal surface
	gm.	*cc.*	%	*cc.*	%	*hrs.*	
13	1340	4	0.1	3.0	0.1	1	Barely perceptible, staining uniform in distribution
38	1930	8	1	4.1	1	3	
45	2000	10	1	5.0	1	5	Somewhat deeper staining with a suggestion of irregularity in distribution
48	1880	10	1	5.3	1	5½	
49	1500	10	1	6.7	1	5½	
53	1290	10	1	7.8	1	8½	
54	1265	10	1	7.9	1	16	Well marked staining with definite differences in the intensity of colour in different areas. Distribution of variations in intensity were charted
35	1400	10	1	7.1	1	19	
39	2140	8	1	3.7	1	23	
40	2020	8	1	4.0	1	23	
12	1770	5	1	2.8	1	24	
47	2280	10	1	4.4	1	24	
41	2170	8	1	3.7	1	29	
42	2250	8	1	3.6	1	29	
43	1880	8	1	4.3	1	47	
44	1950	8	1	4.1	1	47	
55	1950	10	1	5.1	1	101	

The depth of staining increased with increasing duration of the experiment and appeared to be dependent upon the time rather than upon the quantity of trypan blue, within the limits of dosage employed. It is, however, worthy of note that there was sometimes a considerable variation in the intensity of staining of the aorta in experiments of equal duration, even when comparable quantities of the dye had been injected—a fact which Hackel seems to have overlooked. With increase of the length of the experiments the contrast between deeply stained and lightly stained areas became more marked. However, beyond twenty-three or twenty-four hours, no obvious change in the character of the staining could be distinguished.

Prior to the appearance of blue colour on the intimal surface of the aorta, staining was to be seen in other tissues, as for example in the subcutaneous connective tissue and the peritoneal surface of stomach and intestines. The lungs, liver, spleen and kidneys were also tinged

with blue before staining became prominent on the intimal surface of the aorta. In the kidney the staining was considerably deeper in the cortex than in the medulla.

In the aorta blue colouring was first seen in the sinuses of Valsalva, forming a distinct ring around the circumference of the vessel. Staining was also well marked along the borders of attachment of the aortic valves. The valve leaflets were, however, only slightly tinged. The pulmonary valve ring was similarly stained. This staining in both the aorta and pulmonary artery, in the early stages, stopped quite abruptly at the upper margins of the sinuses, while the intimal surfaces of both arteries showed practically no colour. In the longer experiments, where the whole intimal surface of the aorta appeared blue, the aortic valve ring was always prominent by reason of its deeper staining.

The earliest distinguishable colour on the intimal surface of the aorta was always uniformly distributed throughout its whole extent. At this stage it was very obvious that the colour on the adventitial surface of the aorta was distinctly deeper than that on the intimal surface. This difference in the intensity of staining of the two surfaces of the aorta was apparent in all the specimens, but in the longer experiments the difference was not so great.

In the longer experiments lasting sixteen hours or more, the intensity of staining of the aorta as seen from the intimal surface was definitely irregular. The deeper staining patches were not sharply demarcated from the surrounding paler areas, but the colour shaded off from one to the other more or less gradually. Examination of the intimal surface with a strong lens revealed uniform staining within any given small area and the character of distribution of the dye could not be accurately described as "fleck-like." The localization of areas of more intense staining had, in general, a similar distribution in all of the longer experiments; and therefore the aortas from these experiments may be described together concerning the more constant characteristics of distribution of the dye.

The intimal surface of the arch of the aorta always showed more marked irregularities in intensity of staining than elsewhere. A deep staining patch was constantly present, extending from the margin of the anterior sinus of Valsalva upward in the longitudinal axis of the vessel toward the mouth of the innominate artery. This patch was usually broader and deeper in colour at the base, narrowing and fad-

ing in its upper part and thus forming a flame-shaped area. It extended for a variable distance upward but seldom reached the orifice of the innominate artery. Occasionally, however, it split at the upper end, continuing as two more diffuse streaks which passed on either side of the openings of the vessels on the convexity of the arch. Another less distinct patch was almost always present above the left posterior sinus of Valsalva, extending upward for a short distance as a flame-shaped area which gradually faded to the vanishing point. Occasionally this area was extended as a diffuse streak or became broader opposite the opening of the innominate artery, fusing in this area with an extension of the patch first described. The area above the right posterior sinus of Valsalva was always paler than that above either of the other two. The orifices of the great vessels arising from the arch were encircled by a narrow pale staining margin, while darker, mottled areas were often seen near them so that the wall of the aorta on the convexity of the arch was always more deeply stained than that on the concavity. These mottled areas usually resolved themselves posteriorly into a broad streak which extended downward on the posterior wall of the descending portion of the arch toward the openings of the first pair of intercostal arteries. Thus the ascending limb of the arch was most deeply stained on its anterior and left lateral aspects, the colour being deepest at the root of the aorta. In the transverse limb the convexity of the arch and its posterior surface showed the greatest intensity of colour, while in the descending portion the posterior wall was most strongly stained.

The thoracic aorta did not show any patchiness of staining but a distinctly deeper staining of the posterior wall of the vessel was constantly present. This area of deeper staining appeared as a broad streak, often forming a continuation of that coming down from the arch. It was continued laterally a little distance beyond the mouths of the intercostal arteries, and then gradually faded to the paler staining of the anterior half of the vessel. The colour of this area was quite uniform and showed no longitudinal streaking within itself. The only variation in the depth of staining was an extremely narrow margin of pallor just below the mouths of the intercostal arteries. The anterior portion of the thoracic aorta was very lightly stained; indeed, this portion of the aorta showed the weakest staining of any part of the vessel, often appearing almost entirely unstained. This area of pale staining occupied the anterior segment of the circumfer-

ence of the aorta but extended slightly further toward the left side than to the right. Its upper limit was approximately the level of the first pair of intercostal arteries, and it extended downward to the level of the diaphragm. In the lower half it was frequently narrower than above and often narrowed gradually to the vanishing point just before reaching the level of the last pair of intercostal arteries.

The abdominal portion of the aorta showed a more uniform staining, though here too the posterior wall of the vessel tended to be more strongly tinged than the anterior. The mouths of the branching vessels were surrounded by narrow zones of paler staining and this was particularly prominent in the distal margins of the vessel mouths forming small crescents of paler staining in these situations.

All the variations in intensity of staining described, with the exception of the pallor around the mouths of branching vessels, could be very easily detected on the adventitial surface of the aorta, being often more distinct on this surface. Cross-section of the vessel and examination of the cut edge with a strong lens showed that the staining was much stronger in the outer layers of the media than in the inner portion. This difference in depth of colour could often be demonstrated even more strikingly by splitting the vessel approximately through the middle of the media and stripping it apart into two layers. The outer layer of the aortic wall then showed well marked staining, while the inner half was almost uncoloured. However, in some of the more deeply stained patches in the arch, the colour extended through the entire thickness of the media to the intimal surface without any perceptible difference in the depth of staining. These findings were confirmed by microscopic examination of thick frozen sections which were mounted without further staining. In such preparations the elastic fibres of the outer portion of the media were seen to be lightly tinged with blue, while the elastic laminae of the remaining part of the media were uncoloured, save for the internal elastic lamina which was faintly tinged. Examination of deep staining patches in the arch showed the elastic fibres to be coloured through the whole thickness of the media, but more strongly so in the outer layers.

In addition to the more or less constant variations in intensity of staining, two specimens (No. 48 and No. 54) showed a single small round patch located on the right side of the ascending limb of the arch about midway between the valve ring and the mouth of the in-

nominate artery. In one case (No. 48) the experiment had been of only five and one-half hours' duration and this area was quite deeply stained, while the remaining parts of the intimal surface showed only slight staining. These patches were much less prominent on the adventitial surface. On sectioning these areas no abnormality was found in the structure of the wall and the staining was most marked in the layers of the elastic fibres nearest the intima, gradually fading toward the periphery.

In four of the animals one or more "spontaneous" arteriosclerotic lesions of the aorta were present as small, discrete, slightly depressed areas in the arch or the upper part of the thoracic aorta. In every instance these lesions were conspicuous because of the almost complete lack of colour in them. They stood out as small, round, white patches against the blue background. There was no greater intensity of staining around the margins of the lesions than elsewhere in the intima. A patch of slightly deeper staining was, however, to be seen on the adventitial surface opposite each of them.

The pulmonary artery became stained in about the same time that was sufficient to produce perceptible staining of the aorta. The intimal surface of the first part of the pulmonary artery was often mottled in appearance, but variations in intensity were not as marked as in the arch of the aorta. The smaller arteries and veins were also coloured, at least in the longer experiments, and in these, as far as could be determined, the staining was uniform. The thoracic duct, if it was stained at all, was not sufficiently coloured to make it any more conspicuous than usual.

In addition to the experiments described above, three rabbits were anaesthetized and the abdominal aorta exposed by a transperitoneal approach. In one animal the aorta was painted with croton oil around its whole circumference for a distance of about 1 cm. In another only a portion of the external surface of the aorta was bared and painted with croton oil. In the third, the aorta was lightly seared along one side with a hot probe. Immediately after operation in each case the rabbit was given 10 cc. of 1 per cent trypan blue in Ringer-Locke solution intravenously, and killed five hours after the injection. In each animal the aorta was lightly stained throughout its extent, except in the region treated with croton oil or cautery, where both the adventitial and intimal surfaces showed a patch of much deeper blue-staining, corresponding to the area in which the

irritant had been applied. Microscopic sections of these areas showed, in the case of the animals in which croton oil had been employed, a marked inflammatory reaction in the adventitia and surrounding structures, evidenced by intense engorgement of fine vessels and a moderate cellular infiltration. Unstained frozen sections showed a very faint staining of elastic fibres with trypan blue throughout the thickness of the media, but most marked in the peripheral portion. In the aorta which had been cauterized there was necrosis of the adventitia and outer layers of the media with evidence of an inflammatory reaction about the necrotic tissue. Unstained frozen sections showed the localization of the dye in the necrotic area and diffusely in the regions immediately adjacent to it.

The aortas of three other rabbits were prepared in the following manner. The animal was killed by the injection of air into an ear vein. The thorax and abdomen were rapidly opened and the aorta carefully dissected out. The aorta was immersed in Ringer-Locke solution previously warmed to 37° C and was carefully opened in the longitudinal axis with a fine pair of scissors, care being taken to traumatize the vessel as little as possible. Blood was washed out of the aorta with warm Ringer-Locke solution and the vessel then placed in a weak solution of trypan blue in Ringer-Locke solution also warmed to 37° C and kept at that temperature in an incubator. In one experiment the strength of the trypan blue solution was 1:10,000. At the end of thirty minutes the vessel was quite deeply stained. The two other aortas were immersed in a 1:20,000 trypan blue solution, one for fifteen minutes and the other for twenty-five minutes. In both of them the staining was of moderate depth and comparable to the strength of staining in the longer experiments with intravenous injection of the dye. In all three aortas the staining was approximately of equal intensity on both the adventitial and intimal surfaces. The depth of colour was uniform throughout the whole extent of the vessel, except for a few pale areas where surfaces had apparently come into apposition with one another and interfered to some extent with the free access of the dye. Also a narrow zone along the cut edges was more deeply stained. However, the characteristic distribution of the dye, as described above for the experiments where intravenous injection had been used, was entirely lacking. Microscopic examination of unstained frozen sections also indicated that

the staining was of a different character. The elastic fibres in the inner and outer thirds of the media were tinged with blue, while the middle third of the media was entirely unstained.

Discussion

In considering the picture of dye distribution, as seen on the intimal surface of the aorta, one must keep in mind that the aorta of the rabbit is quite translucent and that staining of the adventitia or external layers of the media is clearly visible on the intimal surface of the vessel. This fact can easily be demonstrated by placing a drop of 1 per cent solution of trypan blue on the adventitial surface of a freshly removed aorta and, after a few moments, washing it off and opening the vessel. The situation of the stain in the adventitia can be detected with ease from examination of the intimal surface.

The results of the present experiments indicate that the colour seen on the intimal surface of the rabbit's aorta after intravenous injection of trypan blue is due to dye which is confined chiefly to the adventitia and outer portion of the media or which diffuses inward from this source. This is evidenced by the earlier appearance of colour in the adventitia and by the fact that the former is more deeply tinged, especially in the shorter experiments. Also the variations in intensity of staining seen on the intimal surface of the vessel are equally well marked on its external aspect. Splitting of the aortic wall approximately through the middle of the media and stripping it apart into two layers shows strong staining of the outer half, while the inner layer is almost uncoloured. Furthermore, examination of cross-sections of the aorta, both in the gross and microscopically, shows the presence of the dye in the adventitia and outer portion of the media while the inner layers are relatively unstained. In some of the very deeply stained parts of the aorta, the dye has tinged the whole thickness of the media, but even in these areas the staining is stronger in the external layers.

In view of these facts, any explanation of the variations in intensity of staining, as seen on the intimal surface of the aorta, must be based upon local peculiarities in the structure or function of the adventitia and peripheral third of the media. The experiments in which freshly removed aortas were soaked in weak solutions of try-

pan blue demonstrated that the irregularities of staining in the aortas of animals injected intravenously with the dye were not due to the presence of unusually spongy tissues or to areas possessing a greater affinity for the dye. They indicated, on the contrary, the importance in the staining process of the maintenance of normal blood flow and pressure, not only in the lumen of the aorta, but also in the vasa vasorum.

Impressed by the importance of blood pressure, particularly in the lumen of the aorta, Okuneff, Glasunow and Hackel attempted to establish that the staining is due to penetration of the dye directly from the lumen of the vessel into the intimal surface. They believed that the local variations in intensity of staining could be explained by corresponding variations in the permeability of the lining endothelium which, in turn, was looked upon as being dependent upon variations in the strength of the normal lymph flow from the lumen of the aorta to the lymph channels in the peripheral coats of the vessel. If this were the case, one would expect to find the innermost layers of the aorta stained earliest and most deeply. It is true that in these experiments the internal elastic lamina was lightly tinged by the dye, indicating that some penetration from the lumen did occur, but the staining of this membrane was always diffuse and completely overshadowed by that of the external layers of the vessel wall. It was in the latter that the variations in intensity of staining occurred which produced the irregularities in depth of colour seen on the intimal surface.

From an anatomical study of the aortas of dogs, lambs and humans, Robertson [10] has minutely described the distribution and abundance of the blood supply to the walls of the arch and thoracic portion of the aorta, through the fine vessels which ramify in the adventitia and penetrate the outer third of the media. A comparison of his results with those of the present experiments shows a close correspondence between the depth of staining in the aorta and the vascularization of its walls: the colouring is deepest where the vascular network is most abundant. He found a rich network of vessels about the aortic valve ring and the root of the aorta, but fewer in the ascending limb of the arch. Furthermore, the vascular supply was not equally plentiful around the whole circumference of the vessel. "At the root of the aorta, vascularization was most abundant over the anterolateral aspect and least abundant behind, over the right pos-

terior sinus of Valsalva. . . . The vascularity of the arch was greatest on the convex surface of its ascending portion, and on the posterior surface of its transverse portion. It was least vascular on its anterior surface, particularly toward its descending portion. This latter section was most vascular on its posterior aspect, resembling the descending thoracic limb in this respect." The thoracic portion of the aorta was most richly supplied on its posterior wall, while the anterior segment had a much less abundant vascular network. The similarity between the position of these areas of abundant vascular supply and that of the areas most deeply stained by trypan blue in the present experiments need hardly be further enlarged upon to indicate the probability that the distribution of the dye was dependent upon the abundance of the vasa vasorum in the adventitia and outer third of the media. Where the vascular network was sufficiently abundant the stain was diffused through the thickness of the media, even reaching the inner surface. Apparently the endothelium of the capillary network was much more permeable to the dye than was that of the intimal surface of the aorta and the large vessels. This fact resulted in the appearance of the dye in other tissues, as well as in the external coats of the aorta, at a time when the intimal surface of the aorta was still almost uncoloured.

In two rabbits (No. 48 and No. 54) a small round patch was found on the right side of the ascending limb of the arch of the aorta and in these areas the staining was found to be most marked in the inner layers of the media. These patches probably marked the position of minute nutrient vessels entering the media directly from the lumen of the aorta and ramifying in its inner portion. The presence of such vessels in this same position in dogs has been reported by Woodruff [11] and by Robertson, and the latter also found perforating nutrient vessels of this type in human aortas. In some cases these vessels anastomosed with the network in the outer coats of the aorta, while in others they ended in a ramification in the inner third of the media. The occurrence of such minute vessels probably accounts for the patches observed by Okuneff in the aortas of some of his rabbits and guinea pigs.

An inflammatory reaction induced in three rabbits around a small portion of the abdominal aorta through the agency of croton oil or cautery resulted in earlier and stronger staining in the inflamed areas than elsewhere in the vessel wall. This phenomenon is readily ex-

plained by the local increase of capillary permeability in the inflammatory reaction. That the permeability of the capillary walls to trypan blue does increase in an area of inflamation has been demonstrated by Menkin [12, 13, 14] in experiments upon rabbits and frogs. He also showed that the dye injected into the blood stream rapidly accumulated in an area of inflammation and was fixed there by occlusion of the regional lymph channels and the formation of a network of fibrin around the inflamed area.

Since the greater part of the blue colour seen in the intimal surface of the aorta was imparted to it by the outer coats of the vessel, any local thickening or density of the medial coat or intima would result in an area of relative pallor. Thus a narrow zone immediately below or completely encircling the orifice of each branching vessel appeared paler in colour, due to the presence of a lip-like thickening around the mouth of each arterial orifice, particularly prominent around the distal margin. The failure of "spontaneous lesions" to show any colour on the intimal surface probably likewise depended upon the density of the areas of degeneration in the media which frequently showed calcification. On the other hand, the slightly deeper staining on the adventitial surface opposite such lesions probably was the result of an increased vascularity in response to a degeneration and mild inflammation of long standing.

From the results of the present experiments, conclusions such as have been drawn by Okuneff, Glasunow and Hackel would be quite unjustified. The irregularities in intensity of staining of the aortic wall cannot be said to be due to local variations in the permeability of its lining endothelium. If the behaviour of trypan blue be comparable to that of lipoid materials in the plasma, then one must conclude that local variations in the permeability of the intima to lipoids do not exist under normal conditions, and hence can have no bearing upon the initiation of local fatty deposits in the aorta. The explanation of the origin and localization of such fatty changes must be sought elsewhere.

Conclusions

1. In rabbits, intravenous injection of a suitable quantity of a solution of trypan blue results in well marked staining of the wall of the aorta within sixteen hours. The depth of colour as seen on the intimal surface is not uniform, some areas being more deeply stained

than others. The differences in intensity of staining become more prominent with increase in the length of the experiment.

2. The variations in depth of colour seen on the intimal surface of the aorta are the result of irregularities in the staining of its outer layers — the adventitia and outer portion of the media. The deeply staining areas correspond to the areas in which the aortic wall is most plentifully supplied by vasa vasorum, while the pale staining areas correspond to those in which the vascularization of the aorta is least abundant.

3. The staining of the wall of the aorta is chiefly due to the escape of the dye through the capillary endothelium which is much more permeable to trypan blue than is the lining endothelium of the aorta. The local variations in depth of staining in the aorta are thus dependent upon the degree of vascularization of its walls.

4. The production of an inflammatory reaction in the external layers of the aorta brings about a local increase in capillary permeability to trypan blue and as a result a stronger staining of the vessel wall in the inflamed area.

In conclusion I wish to express my thanks to Professor Oskar Klotz for his interest and advice throughout the progress of this work.

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THE EFFECT OF CABBAGE FEEDING ON THE MORPHOLOGY OF THE THYROID OF RABBITS *

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The production of goiters in rabbits by cabbage feeding reported by Chesney, Clawson and Webster,[1-5] and confirmed by Marine, Baumann and Cipra[6] seemed to introduce an easy and certain experimental means for studying thyroid hyperplasia in rabbits. Although these goitrous rabbits showed heat production lower than normal,[2] yet when iodine was administered to such rabbits there was a striking rise in basal metabolic rate, deposition of colloid, loss of weight and sometimes death.

It was for the purpose of studying carbohydrate metabolism during the hyperthyroid state induced by the administration of iodine to such goiter-bearing rabbits, that a group of rabbits was placed on a cabbage diet.

The diet used by the Johns Hopkins investigators was a daily ration of approximately 250 gm. of cabbage, and a weekly ration of approximately 20 gm. of hay and 50 gm. of oats.[5] The diet used by those working at Montefiori Hospital was 60 calories of cabbage per Kg. daily (equivalent to about 180 gm. cabbage per Kg.), 35 gm. whole oats weekly, and 20 gm. alfalfa hay weekly.[7]

Marine, Baumann and Cipra[6] had found that boiling or steaming the cabbage greatly increased its capacity to produce hyperplasia. Whereas rabbits fed fresh cabbage developed palpable thyroids in about 30 days, the same amount of steamed cabbage produced an equivalent enlargement in 10 to 15 days. Steamed cabbage in amounts as low as 25 calories per Kg. (or about 75 gm. per Kg.) per day produced hyperplasia. They considered that cabbage contained a "powerful goiterogenic agent."

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Experimental Procedure

The rabbits in the experiments reported in this paper were, at the outset, placed on a cooked cabbage diet, as it was anticipated that a shorter period of feeding would be required to develop goiters.

The feeding was started in January 1931, with 17 rabbits. White winter cabbage was used. That used for the first two months was grown in New York state. For the first month the animals were fed 35 gm. whole oats weekly, 20 gm. hay weekly, and as much cooked cabbage as they would eat.

Four animals died during this period after losing weight rapidly. Three of these animals showed hemorrhagic lungs at autopsy.

After the first month, as the animals were not gaining weight on this diet, the cabbage of each rabbit was weighed daily, and the oats increased to approximately 50 gm. The cabbage was steamed in the autoclave (at first under pressure, but later no pressure was used) and was given in the amounts indicated in Table I. The weights recorded are for the cooked cabbage after steaming. The animals ate voraciously and seemed hyperactive, and when excited sometimes showed protrusion of the eyes. As much cabbage was given to the animals as they would eat, and from the table it will be seen that the amount eaten was much more than was given by either the Johns Hopkins investigators or by Marine and his co-workers. However, only 5 animals maintained their original weight, as may be seen from the table, and these 5 did not gain as much as a rabbit would on a regular diet.

At the beginning of the third month, as the animals were still doing poorly and showed no palpable thyroid enlargement, they were divided into two groups, including an equal number of rabbits who were losing weight in each group. This division is indicated in the table as "2nd period" of the experiment.

One group was fed cooked cabbage from which the juice had been expressed in a press. Marine and his co-workers[6] had shown that the juice does not contain the goiterogenic agent, and the pressed cabbage is just as effective as the whole. The cabbage was pressed in order to reduce the polyuria which rabbits show on a cabbage diet. It was thought that this increased water intake might be a factor in producing the poor nutritional state of the animals. As much of this pressed cabbage was given each animal as it would eat, and the

amounts are recorded in Table I. The weights recorded are of the cabbage after expressing the juice, corresponding roughly to 0.7 of the original weight of the cabbage. The second group was fed raw cabbage.

At Montefiori Hospital all of the rabbits were of a single strain (Belgian). At Johns Hopkins various types of rabbits were used. In the experiments reported in this paper various types were used.

Results

1. *Condition of Animal:* It will be seen from Table I that 6 animals at the end of their feeding periods had maintained their original body weights, and of these, 3 had exceeded their original weights. Eleven animals were below their original weights, either at spontaneous death or when the experiment was intentionally terminated. The animals studied by Chesney, Clawson and Webster [1] usually gained weight and had no tendency to diarrhea. Some of their animals, however, lost weight rapidly for several weeks and died, the autopsy revealing no cause for death. Webster and Chesney [5] reported a high incidence of intercurrent infection at times. The animals of the present series, which lost weight rapidly, were suffering from diarrhea. Five animals died spontaneously, and 2 were killed because they were ill. In 4 ill rabbits, there were acute inflammatory lesions in the lungs. The rabbits used were not a parasite-free breed, and coccidia were sometimes found at autopsy.

2. *Weight of Thyroid:* The only thyroids in the present series that were distinctly larger than normal were one of 0.641 gm. after 102 days of feeding (R73), and one of 0.4398 gm. after 107 days (R64). Comparable weights were obtained by Chesney, Clawson and Webster [1] in much shorter periods of time. For instance, they obtained 0.86 gm. as the average weight for all rabbits observed for 41 to 60 days, and 0.43 gm. as the average of all animals observed for 21 to 40 days. Their data for periods of observation comparable to mine show that at 101 to 120 days the minimum weight of the thyroid was 0.4 gm., the maximum 3.0 gm., and the arithmetical mean 1.47 gm. For 81 to 100 days they obtained a weight of 0.1 gm. as a minimum, 2.3 gm. as a maximum, and 1.05 gm. as an arithmetical mean.

Marine[7] reported externally palpable thyroids and enlargements up to two and two and one-half times on direct observation in 4 rabbits fed 81 days on raw winter cabbage (1928); palpable thyroids and enlargements of three to four times in 4 rabbits fed 56 days (1928); palpable thyroids and enlargements of twice normal size in 8 rabbits fed 28 days (1928); and palpable thyroids and enlargements of three times normal size in 2 rabbits on the diet 22 days (spring of 1929). During the fall and winter of 1929–1930 he reported that cabbage was less goiterogenic, that is, the thyroids could not be palpated after 21 days of feeding. These figures were for raw cabbage.

Comparison of our data with that of previous investigators shows that under the conditions of the experiment reported in this paper 1931 cabbage produced very little gross enlargement of the thyroid. The three heaviest thyroids were obtained in rabbits which had been transferred from a cooked cabbage diet to raw cabbage.

3. *Microscopic Appearance of the Thyroid:* In the papers from Johns Hopkins,[1,3] microscopic descriptions have been given of the goiters in a stage of advanced hyperplasia, and descriptions have been given of the microscopic changes of involution induced in these enlarged thyroids by iodine administration. The early stages in the formation of the goiter, however, were not described. In the present paper, therefore, the microscopic changes will be considered in some detail, as they apparently represent the early stages in the development of the type of goiter which results from a cabbage diet up to the stage at which the other observers began their microscopic studies.

Although the gross changes in the thyroid were not striking in the present series of experiments, the microscopic changes were pronounced and represented an interesting transition from the normal to the hyperplastic.

The microscopic slides were arranged in order of what was considered the degree of microscopic change, without referring, until after the histological description, to the data of weights and duration of thyroid feeding. In Table I the rabbits have been arranged in this order, ranging from Group I in which the microscopic changes were slight, to Group III in which the microscopic changes appeared to be the most advanced in this series.

Group I. Early Changes: The essential changes were thinning and disappearance of the colloid, change in shape of the epithelial cells, and increase in number of the epithelial cells, with formation of new small acini. In some thyroids the change in the epithelial cells far exceeded the disappearance of colloid. That is, the former flat epithelial cells became oval and rounded, even when enclosing a large amount of fairly dense colloid which, however, had become paler than normal. Where the colloid was most dense and eosinophilic, the cells remained very flat. In addition, there were solid masses of round and oval epithelial cells with abundant cytoplasm lying in between large acini and grouped together without apparent formation of acinar spaces. These changes are exemplified in R51 and R59 (Fig. 1).

In R58 there was more marked change in the colloid than in the epithelial cells. The colloid had become pale and granular. Apparently in life this colloid was quite fluid, as it seemed to have sedimented by gravity, and had thus formed crescents of eosin-staining material in each acinus with an upper zone of granular, poorly staining material, while above this the acinus was empty. There was relatively little rounding of the epithelial cells, but there was some new-formation of epithelium between old acini.

In other cases (R62, R61, R66) thinning and disappearance of colloid occurred almost simultaneously with rounding of the cells and new-formation of epithelial cells. Sometimes a few acini containing dense eosinophilic colloid stood isolated, surrounded by acini containing pale, fluid colloid (Fig. 2). Whenever there was evidence of thinning of colloid, dense crescents were found in the acini with granular colloid above. The acini became somewhat smaller in size and new epithelial cells appeared between acini. The changes in this group were only slightly beyond physiological variations, but in no instance was the "resting" colloid stage seen.

In 2 of the rabbits that died in less than a month (R65 and R60) the microscopic appearance did not correspond with the sequence of changes in the other thyroids, and possibly other factors complicated the picture. In these, pale colloid not only distended the acini but occurred in lakes outside the acini. There appeared to be ruptures in the acinar walls, permitting this flowing out of colloid into the interstitial spaces.

TABLE I

Data on Cabbage-Fed Rabbits. (Group I, Showing Slight Microscopic Changes)

Animal No.	Body weight, Kg. Original wt. Period		Body weight, Kg. Final wt.	No. days	Cabbage feeding: Approximate No. gm. per day	Cabbage feeding: Condition of cabbage	Thyroid: Weight gm.	Thyroid: Gross	Thyroid: Microscopic	Condition of animal
R51	1st 2nd	2.68 2.48	2.48 2.32	70 43 113	450–700 500–700	Cooked, not pressed Raw	0.1284	Small, pale	Epithelium flat and cuboidal. Most acini large, a few small. Colloid abundant and mostly dense	Snuffles during first month. Steady loss of weight throughout experiment
R59	1st 2nd	2.38 2.10	2.10 2.12	70 44 114	450–700 400–700	Cooked, not pressed Steamed, pressed	0.1010	Small, pale	Epithelium becoming cuboidal. Many small acini. Colloid abundant and dense	Good condition
R58	1st 2nd	2.34 1.98	1.98 1.50	70 39 109	450–700 450–600	Cooked, not pressed Steamed, pressed	0.1190	Small, pale	Epithelium quite flat, some cells cuboidal. Acini large and medium sized. Colloid pale and granular, with formation of crescents	Steady loss of weight. Killed as appeared ill. Intestine contained no solid feces
R62	1st 2nd	2.10 2.02	2.02 2.06	63 44 107	400–700 400–700	Cooked, not pressed Steamed, pressed	0.1655	Small, vascular	Epithelium becoming cuboidal. Many small alveoli. Increase in cells between acini. Colloid pale and granular, with crescents	Good condition
R61	1st	2.44	1.50	36	600	Cooked, not pressed	...	Very small, congested	Epithelium flat and cuboidal. Some small acini. Increase in cells between acini. Colloid granular, with crescents	Killed because ill. Diarrhea and loss of weight. At autopsy, intestine contained no solid feces. Coccidia
R66	1st	2.04	1.50	33	ad lib	Cooked, not pressed	...	Very small, pale	Epithelium flat and cuboidal. Many small acini and oval epithelial cells between acini. Colloid scanty and granular	Found dead. Left lung hemorrhagic. Intestine contained no solid feces. Coccidia
R65	1st	2.66	2.26	17	ad lib	Cooked, not pressed	...	Small, slightly congested	Mostly flat epithelium. Small and medium sized acini. Colloid pale but homogeneous and has escaped outside acini	Found dead. Hemorrhagic lungs
R60	1st	2.44	2.24	24	ad lib	Cooked, not pressed	...	Small, slightly congested	Mostly flat epithelium. Scanty granular colloid, some outside acini	Found dead. Hemorrhagic lungs. Blood-stained fluid in chest and peritoneal cavities

								slightly congested	lar colloid, some outside acini	Blood-stained fluid in chest and peritoneal cavities

(*Group II, Showing Microscopic Changes of Moderate Degree*)

R71	1st	2.16	1.72	24	ad lib	Cooked, not pressed	....	Very small, slightly congested	Epithelium cuboidal. Very cellular areas where lumina not discernible. Scanty granular colloid	Found dead. Intestine contained no solid feces
R57	1st 2nd	2.30 1.90	1.90 2.06	70 24 94	450–700 450–500	Cooked, not pressed Steamed, pressed	0.2520	Pale	Cells cuboidal. Very little new formation of acini. Scanty granular colloid	Found dead. Lungs consolidated, pus in pleural cavity
R69	1st 2nd	2.18 2.26	2.26 2.24	63 39 102	400–700 600–700	Cooked, not pressed Raw	0.1415	Pale	Cells all cuboidal. Large acini and new small acini. Colloid granular	Good condition
R67	1st 2nd	1.96 2.00	2.00 1.96	63 42 105	400–700 400–600	Cooked, not pressed Steamed, pressed	0.0882	Vascular	Cells cuboidal. Large acini as well as new small ones. Colloid granular	Good condition
R72	1st 2nd	2.44 2.06	2.06 1.96	63 38 101	400–700 600–700	Cooked, not pressed Raw	0.1630	Pale	Cells cuboidal. New acini. Granular colloid with crescents	Loss of weight. Coccidia
R68	1st 2nd	2.42 2.44	2.44 2.58	63 40 103	450–700 400–600	Cooked, not pressed Steamed, pressed	0.1834	Pale	Cuboidal cells. New formation of many small acini. Colloid varies from dense to granular	Good condition

(*Group III, Showing Microscopic Changes of Advanced Degree*)

R63	1st 2nd	2.10 1.82	1.82 1.80	63 40 103	400–700 600–700	Cooked, not pressed Raw	0.2720	....	Extremely cellular. High cuboidal epithelium. Most acini small. Granular colloid where present	Good condition
R64	1st 2nd	2.30 2.28	2.28 2.28	63 44 107	400–700 500–700	Cooked, not pressed Raw	0.4398	Extremely vascular	Extremely cellular. Mostly small alveoli with high cuboidal epithelium. Very little colloid	Good condition
R73	1st 2nd	1.92 2.26	2.26 2.36	63 39 102	400–700 600–700	Cooked, not pressed Raw	0.641	Extremely vascular	High cuboidal epithelium. All acini small. Almost no colloid. Capillaries conspicuous	Good condition

Group II. Changes of Moderate Degree: The thyroids of this group showed acini of moderate size and very small size. The epithelial cells were distinctly cuboidal and there was extensive hyperplasia, as indicated by the dense masses of round cells without discernible lumina lying in between small, apparently new-formed acini. The epithelial cells of the moderate sized acini were very prominent because of the large amount of cytoplasm and clear round nuclei. The colloid was very pale and granular and generally greatly reduced in amount (see Fig. 3 in which the histological changes were quite advanced in a small thyroid).

Group III. Changes of Advanced Degree: In the three heaviest thyroids the epithelial cells were high cuboidal, with a large amount of pale, vacuolated cytoplasm. Most of the cells occurred in dense groups forming very small acini. The colloid was scanty and when present was pale and granular. Capillaries were conspicuous. Fig. 4 represents the most advanced change, which has the appearance of the stage studied by Chesney and Webster.

Discussion

Apparently there is very wide variation in the way in which rabbits respond to cabbage diet, either by microscopic hyperplasia or gross enlargement of the thyroid. Webster and Chesney commented upon individual susceptibility of certain rabbits to the goiterogenic agent, and noticed that goiter was more easily produced during the winter. Just after the present experiment was started, Webster, Marine and Cipra [7] published results showing seasonal variation in the goiter produced by feeding of cabbage, and variation from year to year, that is—1929 cabbage was not as effective as 1928, and 1928 not as effective as 1927. Their results are stated as the beginning of a systematic attempt to study seasonal variations. The present experiments, although limited in number, may add further data indicating annual variation and a failure to produce thyroid hyperplasia of high grade by feeding winter cabbage during the early part of the year 1931, under the conditions of the experiment.

McCarrison [8] has recently reported obtaining goiter in rabbits by cabbage feeding in India. On examining his figures, it is seen

that the degree of hyperplasia was not very great, the heaviest thyroid obtained weighing 0.697 gm.

The experiments reported in the present series are few in number because intended for another purpose, and conclusions must therefore be guarded, and yet in the findings reported in previous years the regularity with which hyperplasia resulted was such as to anticipate hyperplasia in a large percentage of a small series. When the results became apparent, it was no longer possible to obtain winter cabbage of the same year, and therefore extension of the experiments to a larger series was impossible.

Summary

1. Feeding winter cabbage in the early part of 1931 to 17 rabbits for periods up to 114 days produced hyperplasia of the thyroid, but only in two instances resulted in enlargements more than twice the normal weight.

2. The microscopic changes of hyperplasia were more conspicuous than the gross enlargement.

3. Under the conditions of these experiments, the feeding seemed to favor a high incidence of intercurrent infections.

4. The data, in so far as a small series of experiments permit conclusions, support the view that there is annual variation in the goiterogenic agent of cabbage.

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DESCRIPTION OF PLATE

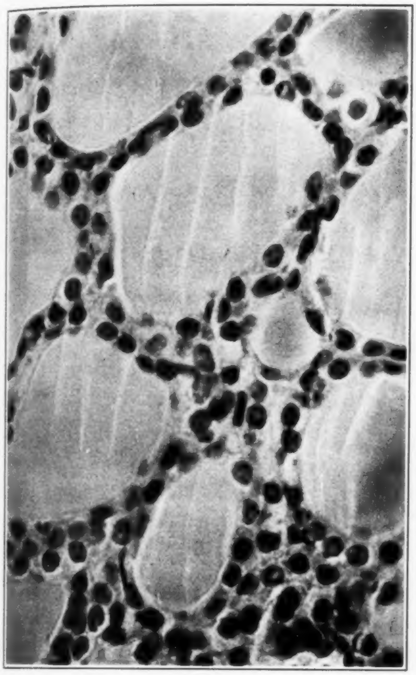
PLATE 37

FIG. 1. Very little histological change in R59 after 114 days of feeding. Thyroid weighed 0.101 gm. × 560.

FIG. 2. R62. Cells are generally low cuboidal. Colloid in some acini is becoming thin and granular, in contrast to a few acini containing dense eosinophilic colloid surrounded by flattened cells. Thyroid weighed 0.1655 gm. 107 days' feeding. × 560.

FIG. 3. R67. Cells are high cuboidal, many acini are small, and the little colloid that remains is granular and pale. Thyroid weighed only 0.088 gm. 105 days' feeding. × 560.

FIG. 4. R73. This represents the most advanced hyperplasia noted, in the heaviest thyroid of the series. The cells are high cuboidal, acini very small, and the colloid has largely disappeared. The thyroid weighed 0.641 gm. 102 days' feeding. × 560.



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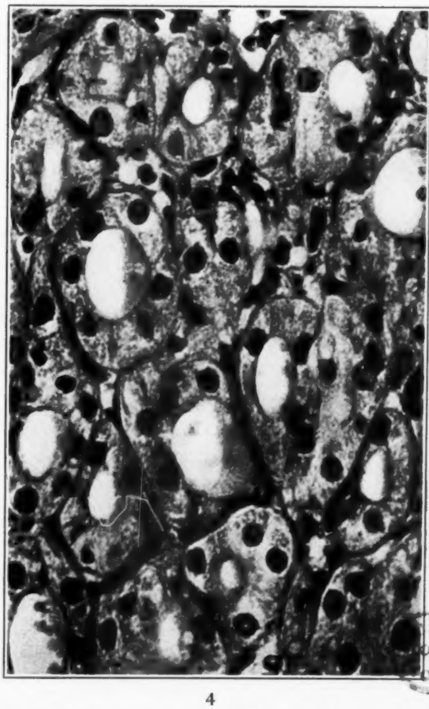

4

Zeckwer

Effect of Cabbage Feeding on Thyroid of Rabbits

A TECHNIQUE OF SILVER IMPREGNATION FOR GENERAL LABORATORY PURPOSES *

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Anyone who has used silver impregnations over a period of time will have been struck with the possibility of applying them to the demonstration of histological elements other than fibers, and will have been tempted to devise some method that would, at one and the same time, bring out these structures as well as the fibrous elements in a given section. There seems to be no reason why a silver impregnation should not be arranged to fit the purposes of routine tissue examination in the pathological laboratory, a method that would be an improvement over the usual routine stains, inasmuch as it would demonstrate a variety of tissue elements selectively without rendering the use of several stains on several sections necessary.

The following method was designed primarily to demonstrate the finer fibrils in tumors of the melanoma group which elude silver impregnation when the usual methods are employed. They could be shown in frozen sections, but only partially or unsatisfactorily brought out in paraffin material. We therefore experimented with a series of some thirty-five different modes of procedure and discovered that it is possible to obtain even better results in paraffin than in frozen sections. While doing this, we were struck with the general applicability of the method to the demonstration of other tissue components as well. It was found that the preliminary bleach with potassium permanganate and oxalic acid, used in prevailing methods for impregnating reticulum and endoneurium, was the stumbling block that had obstructed successful impregnation of the finer fibrils of our tumors. Further experimentation demonstrated that a preliminary treatment with pyridin and glycerol, in place of the bleach, was practically essential for the attainment of satisfactory results and, in subsequent work, the use of a reducing agent following the gold toning bath (as suggested by Laidlaw's work[1,2])

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was found to be of exceptional value in sharpening the details of the impregnation and converting it essentially into a double impregnation. There are six variants of our procedure chosen from our experimental series of thirty-six variants. The reader need not be alarmed at this large number of variants: it is intended that they shall be used to fit the case in question and ample indication will be afforded for the choice of the proper one, with a tabular view of the results obtainable with each. Unless the best one for a particular purpose be chosen, the results will not be optimal, although any one of the six will give pictures superior to those obtained through methods heretofore used for demonstrating the finest fibrils of the connective tissue. The method is simple, counterstaining is entirely eliminated, and every detail of a given tissue may be brought out sharply.

Technique

Fixation: The finest results obtained were seen in sections made from material fixed in formalin and kept as museum specimens in Kaiserling III for nearly ten years. This fixation, however, is scarcely to be considered practical. The next best fixative is neutral 10 per cent formalin, in which blocks cut thin enough to ensure complete penetration of the fluid should remain for 24 hours at least, longer if possible. If Bouin's fluid is used, the results are comparable to those obtained in the Laidlaw-Bouin method; the nuclei will be unimpregnated, the cytoplasm impregnated in the case of epithelial cells, and mesoblastic cells will be unstained. The resulting pictures are more colorful than those obtained by the Laidlaw procedure.

The method gives very good results if Zenker-fixed tissues are used. They should be fixed for 24 hours, washed in running water for another 24 hours and, after embedding and sectioning, the mercuric chlorid should be removed from the sections with the usual alcoholic iodin solution, and this in turn removed with very weak (1 per cent or less) aqueous sodium thiosulphate. This must then be washed out thoroughly. The oxidation-reduction steps, in which potassium permanganate and oxalic acid are used, *should be omitted* as they produce effects similar to the Bouin fixation. The presence of chromium salts makes no material difference in the subsequent impregnation, except to enhance the impregnation of nervous tissue. On the whole,

formalin fixation gives more colorful results and is, on this account, to be preferred. This does not, however, imply that Zenker fixation is to be eschewed — quite the contrary; it gives very striking pictures in all instances and is well suited to the method.

Embedding: The ordinary routine method of paraffin embedding is used after dehydration of the tissue in ascending percentages of alcohol and in chloroform.

Preliminary Treatment: This is essential in the case of all the variants. The sections are deparaffinized in 2 changes of xylol and absolute alcohol and are then treated from 1 to 24 hours with a mixture of 2 parts pure pyridin to 1 part of pure glycerol. This bath keeps well and may be used repeatedly for many weeks. The sections are transferred directly from this to 2 changes of 95 per cent alcohol, washed in tap water and placed in distilled water.

Impregnating Fluid: This is a simple silver diammino hydroxid solution, depending upon the Kubie and Davidson formula.[3] It is used in all the variants, at full concentration in the first three, at half strength in the last three. To 10 cc. of 10.2 per cent silver nitrate solution in distilled water, strong ammonia is added dropwise until the resulting brown precipitate is just dissolved; 10 cc. of 3.2 per cent pure sodium hydroxid solution in distilled water is added and the reprecipitated silver hydroxid again just dissolved by the addition of a few more drops of ammonia. The solution is then made up to 100 cc. with distilled water that has been heated to about 50°C. Sections are impregnated in this in a closed staining box in the incubator at 37° C, or the paraffin oven at 55° C for 1 hour in the case of Variants 1, 2 and 3, and for 10 minutes in the half-strength solution (5 cc. silver nitrate, 5 cc. sodium hydroxid) in that of the other three variants.

Silver diammino carbonate may be used interchangeably with, and in the place of the hydroxid; it often gives superior results, particularly in those variants in which the tannate mordant is used. It is made up at full strength in all cases; 10 cc. of 10.2 per cent silver nitrate, strong ammonia drop by drop until the precipitate is dissolved, and 10 cc. of 3.1 per cent sodium carbonate in distilled water, instead of the hydroxid. There is no reprecipitation upon adding the carbonate, as the hydrogen ion concentration remains unchanged, and further ammonia is therefore unnecessary. The solution is used in exactly the same manner as the hydroxid.

Reducing Fluid: The developer is a mixture of strong neutral formalin (40 per cent formaldehyd) 1 cc., 1 per cent sodium carbonate in distilled water 3 cc., and distilled water to make 100 cc. Three minutes completes reduction.

Toning and Fixing: The toning bath is a 1:500 solution of Merck's "acid brown" gold chlorid in distilled water. The fixing fluid is the usual 5 per cent sodium thiosulphate ("hypo").

Variant 1

The sections are taken from distilled water, impregnated for 1 hour in the impregnating fluid, washed in 2 changes of distilled water and reduced in the developer for 3 minutes or so. They are then washed in tap water and toned for 3 or more minutes in the gold bath, washed, and fixed in the hypo for 3 or more minutes, after which they are washed and mounted in Canada balsam, after dehydrating in ascending percentages of alcohol and xylol.

Variant 2

This is similar to the preceding formula, except that the Laidlaw oxalic acid (5 per cent) bath is intercalated between the toning and fixing baths, and the fact that toning, redevelopment and fixing are all lengthened to 10 minutes each, to correspond with Laidlaw's directions.

Variant 3 *

In this variant formalin-soda replaces the oxalic acid procedure of its predecessor. It is made up exactly as before (formalin 1 cc., 1 per cent sodium carbonate 3 cc., distilled water to 100 cc.). Used developer should not be employed; it should be made up freshly each time. The treatment with the gold, formalin and hypo is the same as in Variant 2.

Variant 4

In the following three variants a tannic acid mordant is used made up as follows: pure tannic acid 0.2 gm., ammonium bromid 3.5 gm., strong neutral formalin 5 cc., distilled water to make 500 cc.

The sections are mordanted for 15 minutes in the tannic acid bath

* Instead of the soda-formalin solution a solution of 0.5 per cent oxalic acid in 5 per cent neutral formalin has been found to give better results and avoids the danger of precipitates. This was ascertained since the paper was submitted for publication.

TABLE I

Color Variations in Tissues Stained for Silver by 6 Variants

Tissue	Variant 1	Variant 2	Variant 3	Variant 4	Variant 5	Variant 6
Nuclei	Brown	Magenta, slightly brownish	Dull magenta-brown	Brown or black	Black	Sharp brown, reddish or black
Epidermal cytoplasm	Slate brown to brown	Slate violet to magenta	Rose slate, brownish to magenta	Slate brown to fuscous	Violet to violet-brown	Slate blue to slate brown
Glandular cytoplasm	Slate brown	Slate brown to magenta	Magenta-gray	Pinkish gray to brown	Pinkish gray to violet	Violet brown
Erythrocytes	Brown	Magenta	Dark brown to black	Reddish brown	Violet-brown	Brown to seal brown
Collagenous fibers	Lilac to light magenta	Deep magenta to violet	Dull magenta	Pinkish red to magenta	Magenta to scarlet-magenta	Brick red
Reticular fibers	Black	Black	Dark magenta to black	Pinkish red to magenta	Magenta to scarlet-magenta	Brick red
Endoneurial fibers Meissner's nevus cells	Red to black	Magenta to black	Magenta to violet or black	Red to black	Magenta to black, finest often carmine	Brick red
Skeletal muscle fibers	Slate brown striæ black	Magenta to dark red, striæ red to brown	Slate pink, striæ red to brown	Pinkish brown striæ black	Violet, striæ deep magenta	Violet to black, striæ indistinct, too intense
Cardiac muscle fibers	Gray	Magenta-gray	Slate pink	Gray, striæ blackish	Violet, striæ magenta	Violet, striæ magenta
Smooth muscle fibers	Gray	Magenta	Rose-gray	Pinkish to brownish gray	Violet	Slate violet
Myelin sheaths	Black	Black	Black	Pinkish red to magenta	Magenta to scarlet-magenta	Brick red
Nerve trunks	Pink to red	Magenta	Magenta	Brownish pink, epineurium darker	Magenta, epineurium darker	Brick red, epineurium grayish
Melanin	Black	Blue-black	Black	Black	Blue-black	Black

heated to 50° C in the incubator or paraffin oven. They are then treated for ½ to 1 minute with 100 cc. of distilled water to which has been added 3 to 5 drops of strong ammonia. This is the "stop" solution. They are then washed for about 2 minutes in distilled water. The impregnation with silver is complete at the end of 15 minutes instead of 1 hour, as in the preceding variants. After impregnation the sections are washed in distilled water, developed, toned and fixed as in Variant 1.

Variant 5

Proceeding as in the preceding variant, the method changes as soon as the toning bath is reached, to correspond with Variant 2, lengthening the time to 10 minutes and using the 5 per cent oxalic acid-gold developer in exactly the same manner.

Variant 6

This resembles Variant 5 in every particular save one, formalin-soda developer replaces the oxalic acid bath, as in Variant 3.

The formalin-oxalic acid intensifier may be used here, as in Variant 3.

Summary of Steps in the Variants

1. Neutral formalin or Zenker fixation.
2. Paraffin embedding.
3. Pyridin-glycerol pretreatment for 1 to 24 hours.
4. In Variants 4, 5 and 6; tannic acid mordant for 15 minutes, followed by "stop" solution of ammonia for 30 seconds.
5. (*a*) Variants 1, 2 and 3; impregnation in warm silver diammino hydroxid for 1 hour.

(*b*) Variants 4, 5 and 6; impregnation in this bath at half-strength for 10 minutes.

6. Reduction of silver in formalin-soda developer for 3 minutes.
7. Toning in 1:500 gold chlorid in Variants 1 and 4 for 3 minutes; other variants for 10 minutes.
8. Reduction of gold in Variants 2 and 5 with 5 per cent oxalic acid; Variants 3 and 6 with formalin-soda; in either case for 10 minutes.
9. Fixing in 5 per cent sodium thiosulphate in Variants 1 and 4 for 3 minutes; other variants for 10 minutes.

Note: Thorough washes are indicated between all steps, distilled water being required until the sections have been reduced in Step 6; after that tap water is employed throughout.

Discussion of Results Obtained

After studying many sections from the experimental series used in our work we ran through a set of eleven sections in each variant, the material being taken from ten different organs (heart, two sections of lung, thymus, spleen, liver, kidney, suprarenal, uterus, lymph node and brain) from an autopsy performed almost immediately post-mortem. The color effects of the variants were then tabulated in the appended table. The sixty-six slides of the autopsy series were all impregnated at the same time and therefore represent a standard result.

As will be seen, the intensity of detail progresses through the series up to the fifth variant where it is most marked, and falls off a trifle at the sixth. The use of oxalic acid after toning the sections in gold chlorid develops the partially reduced gold salts that have replaced the silver and thus, by further reduction, "doubles" the impregnation: as a result one sees intensified and predominant magentas and violets, which are "gold colors." The use of a stronger reducer (formalin-soda) changes the picture from a prevalent magenta to a brick red, enhances the nuclear detail, impregnates the cytoplasm less densely, but does not produce as precise a fiber impregnation as does the weaker reducing agent (oxalic acid). This is probably explained empirically by the well known proclivity oxalic acid possesses for reducing gold salts.

It will be noted that the nuclei are listed as being either brown or black in those variants using the tannic acid mordant; there is no transition, they are either the one or the other. This phenomenon doubtless has its significance, occurring as it does in nuclei of the same cell race and apparently similar properties, but just what this may be we do not know. At first it was thought to be an artefact, but this peculiarity has been regularly noted in tannate sections. By referring to the table one may readily gauge the relative merits and drawbacks of the different variants. If it is desired to bring out reticulum selectively, then one of the first three variants should be selected, for the last three are unsuitable as they impregnate collagen

and reticulum exactly alike, magenta or reddish. On the whole, the second and fifth variants will be found to be the best for general use. If the fifth is found to give too intense impregnations, the fourth or the sixth may be substituted. If one desires delicate effects with little or no disturbing cytoplasmic background, then the first variant should be the choice; or the fourth, if a little more cytoplasmic detail, color variety and plasticity are desired. Those variants depending upon the tannic acid mordant will give more colorful pictures, those omitting its use will tend to be monochromatic.

The reader is left to choose the variant that best suits his particular purposes and tastes; we can safely claim that he will find one of them that will fit his needs. Variant 2 gives ideal reticulum impregnation: it is particularly fine in the case of the "Gitterfasern," or reticulum of the liver sinusoids, the lymphoid reticulum and the sheaths of muscles. Variant 5 is particularly suited to the demonstration of muscle striations. With several variants, particularly Nos. 2 and 5, the medulla of the suprarenal is most admirably set off and demonstrated, and the Hassal's corpuscles of the thymus very well brought out because of their metachromatic impregnation.

The four figures in the plate (Figs. 1 to 4) demonstrate the various features of as many typical variants. They were made under exactly similar conditions, except for differences in the time exposure that were contingent upon the density of the impregnation. Four fields, as nearly similar to one another as possible, were chosen in four sections cut from the same block of tissue from a nevus.

It might be well to make some explanation of the different steps used in the six variants. The pyridin-glycerol treatment was originally introduced to increase the definition of the fibrillary structures in nevi, as it was known to be excellent in the case of nerve fibrils and the smaller nevus fibrils were suspected of being such. Whether they are, or not, the pyridin is found to bring them out more clearly than if it is omitted, and to keep down troublesome precipitates. The glycerol was added because it had been noted that Kaiserling III tissue impregnated more colorfully than that fixed in pure formalin. It was found that glycerol did, indeed, increase the metachromasia of the impregnation.

The silver solution is made up equimolar (0.6 molar), which explains the fractions in the "10.2" and "3.2" solutions of silver nitrate and sodium hydroxid. Sodium carbonate is added to the formalin

developer in small quantity to act as a buffer and prevent the formation of formic acid. This promotes complete reduction. Laidlaw specifies tap water in his formalin reducer, but we have used distilled water here in Cincinnati as the tap water has a high chlorin content, owing to the chlorination of the water supply. This point should be borne in mind in other cities where chlorination is the practice and where silver procedures might suffer by having Cl ions present in the water.

The tannic acid mordant is essentially that which is used in the well known Achucarró method for impregnating neuroglia. Its function is to form silver tannate in the sections, thereby shortening the length of time necessary for impregnation and increasing the brown tones in the sections, producing color variety. The ammonium bromid ensures precision and prevents precipitates. The formalin has some effect upon the subsequent impregnation, making it more intense and complete. The "stop" solution of ammonia probably removes excess formalin from the mordanted sections, thus preventing precipitates.

The gold solution replaces the silver in the section with gold. This results in a more pleasing color scheme; without it the sections would resemble Levaditi impregnations, being yellow, brown and black. The oxalic acid, used after toning, reduces the gold that has replaced the silver and intensifies the toning already partially effected in the gold chlorid bath. The use of formalin-soda in place of oxalic acid is attended by similar results, but they are in some ways inferior. The color scheme is more varied, but the details of the finer fibrillary structures are not as clear. On the other hand, it is more suited to the impregnation of the epithelium, for it brings out nuclear detail and impregnates cytoplasm less intensely.

Summary

Six variants of a relatively simple and rapid method for impregnating tissue with silver are described. Although there are a number of steps in the process, they are really very easy to carry out once one has become accustomed to the "schedule." It is not complicated by having six variants, for each of these differs so slightly from its fellows that the change from one to another is a very simple matter, involving little extra time and often materially shortening the total time required. These variants are intended to meet varying needs

and to make the impregnation suitable for general use on almost any tissue. The use of the term "variant" might have been omitted, but it was thought that this clarified the slight variations in the procedure, so we have used that expression in this paper. It is not recommended that the method be used for impregnating brain or spinal cord sections, as there is not enough contrast to make it valuable in that connection.

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DESCRIPTION OF PLATE

Plate 38

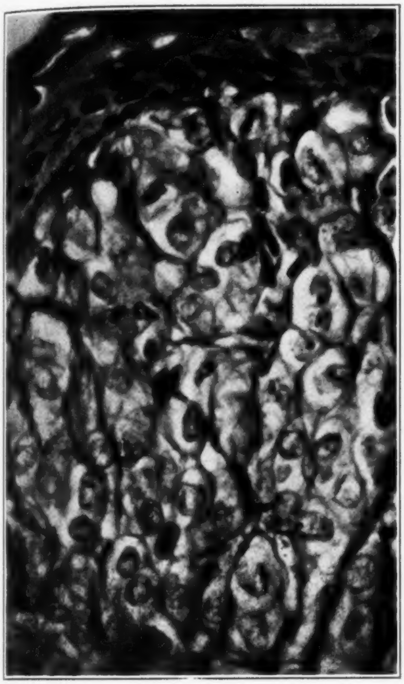
All photomicrographs were taken at about 800 diameters magnification by Mr. Joseph B. Homan of our Department of Medical Art, with the assistance of the authors.

Fig. 1. A field from a pigmented mole, or nevus, impregnated by the first variant. The nevus cells and fibrils are rather pale, the reticulum somewhat darker.

Fig. 2. Similar field impregnated by the second variant. The nevus fibrils are darker, the nuclear detail sharper.

Fig. 3. A third field impregnated by the fourth variant. Nuclei and fibrils still sharper. Note the occasional black nuclei.

Fig. 4. A field slightly deeper in the tumor, but otherwise identical with the preceding. Here the fifth variant was used. The excellent fibril and nuclear detail is at once apparent.



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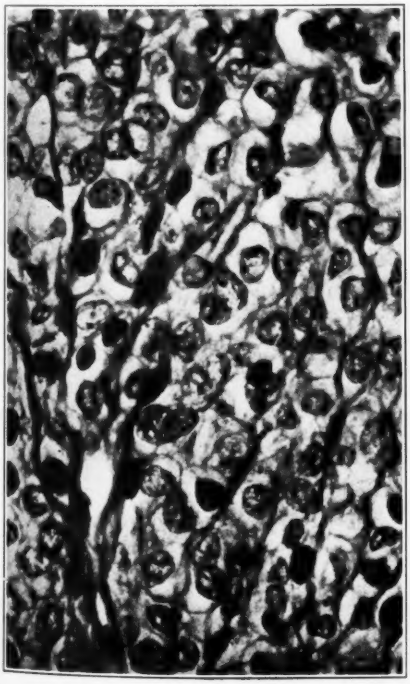

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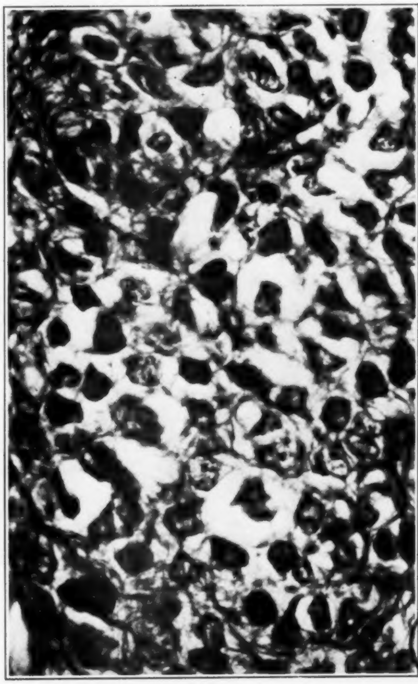

4

Foot and Foot

Silver Impregnation for General Purposes

THE QUESTION OF A SPECIFIC MYOCARDIAL LESION IN HYPERTHYROIDISM (BASEDOW'S DISEASE)*

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Reports in the literature are not consistent in describing morphological changes in the heart specifically related to hyperthyroidism. This variability can be attributed in part to the types of cases examined, to geographical situation, to method and duration of treatment, and to thoroughness of pathological examination. Fahr [1] in 1916 reported in 7 cases (5 of Basedow struma and 2 of colloid struma) degeneration and chronic inflammatory changes in the myocardium; again in 1921 [2] he reported similar changes in a total of 27 cases (18 of Basedow struma, and 9 of colloid struma). These cases were both operative and non-operative. Goodpasture [3] in 1921 reported fairly large foci of acute necrosis in the myocardium of 2 non-operative cases of hyperthyroidism with auricular fibrillation, in which "the cause of death was myocardial exhaustion." Goodall and Rogers [4] in 1927 reported in 9 necropsies of Basedow's disease perivascular and interstitial polymorphonuclear infiltration and patchy necrosis of the myocardium; the changes were present in both ventricles, yet in most of these cases the pericardium, endocardium, valves and myocardium had appeared normal on gross examination.

Other reports, Müller [5] (3 cases), Simmonds [6] (8 cases), Pettavel [7] (4 cases, later 12 in all), Wegelin [8] (13 cases from the Bern Institute, mostly reported by Matti [9] and Pettavel), Wilson [10] (21 cases), Means and Richardson [11] (12 cases), Thomas [12] (1 case), Lewis [13] (12 cases), McEachern and Rake [14] (27 cases), record a variable degree of hypertrophy and dilatation, fatty changes and slight fibrosis. More marked changes, if present, were ascribed to rheumatic heart disease, hypertension or arteriosclerosis. In this group there were no changes considered definitely the result of toxic damage. However, Wegelin found in 1 case necrosis in a papillary muscle on which a

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thrombus had formed. In the group of cases reported from the Lahey Clinic and New England Deaconess Hospital,[13] 1 case with thromboses in both auricles, which were attributed partly to coronary sclerosis, could be considered as developing in the same fashion; another showed evidence of a slight toxic myocarditis.

Two cases are reported here because they present in the myocardium chronic inflammatory changes and degeneration, without complicating factors, and definite enough to indicate the causal rôle of a toxin. The first patient died in thyroid crisis from unabated hyperthyroidism of the Basedow type: there was no operative or known iodine treatment. The second died in a reaction (postoperative storm) following bilateral thyroidectomy: a course of iodine treatment had been given.

Case Reports

Case 1. *Clinical History:* A female patient, 48 years of age, had suffered from struma, palpitation of the heart, exophthalmus, nervousness, and loss of weight for eleven years. Onset of illness followed the death of her father.

Physical examination showed a medium sized woman, incoherent and restless, with nutrition markedly reduced. Exophthalmus present. Tongue damp, cracked and tremulous. Thyroid moderately enlarged, lateral lobes easily palpable. Neck veins prominent. Lungs edematous at bases. Heart action visible, palpable at apex. Palpable pulsation of right ventricle. Heart enlarged by percussion; apex in the seventh intercostal space, three fingers' breadth from mammary line. Heart sounds obscured by indefinite murmurs. Blood pressure 125/75.

Treatment by several courses of strophanthin, glucose solution intravenously, sedatives and rest produced more regular heart action and relieved the dyspnea and delirium. Two weeks later vomiting, looseness of bowel movements, irregular heart action, pleural effusion and edema indicated the bodily collapse of thyroid crisis. The spinal fluid, blood leucocytes, blood sugar and non-protein nitrogen were normal. Blood culture was negative. After two days of marked irregularity and fibrillation of the heart, death occurred in the sixth week following admission.

Anatomical Diagnoses: Struma parenchymatosa (moderate); dilatation of both ventricles of the heart; chronic myocarditis with subendocardial hemorrhage; infarct of spleen (recent); congestion of lungs, liver, spleen, kidneys; edema of lungs; hydrothorax, right; atelectasis of right lung; chronic bronchitis.

Heart: Weight 475 gm. The left ventricle is considerably enlarged, its apex rounded; right ventricle and auricle appear widened. The epicardium is soft, smooth and shining. The foramen ovale

is patent to the extent of admitting a pencil point. The endocardium is smooth and shining. The myocardium of the left ventricle is reddish brown, glazed, and shows small, dark red, hemorrhagic, sunken foci. Under the endocardium of the left ventricle, of the interventricular septum, and of the left papillary muscle are small hemorrhagic foci. The coronaries and valves are negative.

Microscopic Examination: The left ventricle has numerous foci of hemorrhage, mostly under the endocardium and involving the adjacent myocardium; the foci are marked in the papillary muscle. The left ventricle also shows diffuse and focal fibrosis of the myocardium, mostly perivascular, accompanied by diffuse, small, round cell lymphocytic infiltration. There is degeneration of muscle fibers in the foci of hemorrhage, with lymphocytic infiltration and diffuse fibrosis peripherally. These changes are more marked at the distal portion of the small coronary vessels which show congestion and sometimes perivascular hemorrhage. Fat stains reveal diffuse fatty infiltration of muscle fibers, more abundant beneath the endocardium and in the papillary muscle. The larger branches of the coronary arteries are negative.

Sections of the right ventricle reveal moderate diffuse fibrosis, scattered lymphocytic infiltration, and some fragmentation and degeneration of myocardial fibers.

Thyroid: Each lateral lobe is firm and enlarged to the size of a small peach. Microscopically there are numerous follicles, low cuboidal epithelium, abundant colloid, fibrous interstitial bands, and some lymphocytic infiltration. The irregular size of the follicles, degree of involution of the epithelium (spontaneous — no known iodine treatment), and the fairly marked strumitis indicate a hyperplastic process of fairly long duration.

Other Organs: The aorta is smooth and elastic. No thymic enlargement is present.

CASE 2. *Clinical History:* A female patient, 50 years of age, had suffered from enlargement of the thyroid for twenty-nine years. No symptoms accompanied the onset of enlargement; later, there developed increase in size after menstruation. For ten years, especially in past year, there had been palpitation of the heart, headaches, tremor of hands, weakness on exertion, nervousness, excitability, sweating, attacks of diarrhea, and loss of weight. The physical findings accorded with the above symptoms. There was marked undernourishment. There was irregular enlargement in the region of the thyroid. The heart action was rapid, forceful, and irregular; signs of moderate cardiac hypertrophy were present.

The basal metabolism on admission was 57 per cent; after treatment with Lugol's solution, 5 drops 3 times daily for twelve days, it was plus 54 per cent. Lugol's solution was discontinued, and bromural, 0.5 gm. daily, given for three weeks. On bilateral strumectomy, two portions of thyroid (the size of an apple and the size of a walnut) were removed. Following operation the pulse rose from 90 to 140. Marked cardiac irregularity, delirium, tremor and restlessness followed. Despite emergency measures the patient died on the third day.

Anatomical Diagnoses: Status of postoperative partial strumectomy; fatty infiltration of liver; nephrosis (fatty infiltration of renal tubular epithelium).

Heart: Weight 300 gm. Epicardium, endocardium, valves and coronary vessels show no gross abnormalities. Ventricles are well developed. Myocardium is lax and of dull brown color.

Microscopic Examination: Both ventricles have foci of degeneration of myocardial fibers, increase of fibrous tissue, and small round cell lymphocytic infiltration. Capillaries are dilated, partly congested. Small coronary vessels are normal. Fat stains show diffuse fatty infiltration of the muscle fibers.

Thyroid: At autopsy a small distorted portion of tissue was found. The operative specimen consisted of an apple- and a walnut-sized portion of reddish brown tissue, partly encapsulated and showing degenerative changes. Microscopically this specimen consists of many small and medium sized follicles with cuboidal epithelium, some large follicles with low cuboidal epithelium and abundant colloid. There is some papillary hyperplasia, but the stroma between follicles is slight. There are several foci of fibrosis and old hemorrhage. The diagnosis is endemic goiter with secondary follicular hyperplasia and involutional changes.

Other Organs: The kidneys show marked fatty infiltration of the tubular epithelium (nephrosis). There is fatty infiltration of the liver. The spleen is congested; pancreas negative. The aorta is smooth and elastic. No thymic enlargement is present.

Discussion

The explanation of the degenerative and inflammatory myocardial changes involves the question not only of a circulating toxin, but of a toxin apparently acting specifically on the myocardium. Whether the symptomatic effects of hyperthyroidism represent an excessive secretion of normal product of the thyroid gland (hyperthyroidism),

or the secretion of an abnormal and presumably toxic product (dysthyroidism) has not been settled. The view has been generally accepted by clinical investigators that the rôle played by the thyroid is one of hypersecretion. Hyperplasia of the gland is present, therefore hypersecretion. Removal of a portion of the gland, or irradiation, reduces the degree of activity. Involution of the hyperplastic epithelium (through the effect of iodine) accompanies, or is accompanied by, a moderation of symptoms. The ingestion of thyroxine or of dried thyroid gland produces symptoms in man and animals of thyroid overactivity.

On feeding thyroid material to animals, thereby inducing a toxic condition, changes in the myocardium have been observed. Bircher[15] produced goiter in rats by feeding water from goitrous regions. The hearts showed macroscopic hypertrophy, microscopic cloudy swelling and fatty infiltration of muscle fibers, leucocytic infiltration and fibrosis. Farrant [16] found in the hearts of cats and rabbits (fed thyroid tissue), general wasting and hyaline degeneration of fibers, with few nuclei and no transverse striations present. The animals showed a marked general toxic reaction, manifested by rapid loss of weight, bodily weakness and diarrhea. Death of the rabbits occurred after five to nineteen days. In white rats fed thyroxine, Hashimoto [17] found focal myocarditis, later replaced by fibroblasts; these foci resembled Aschoff's rheumatic nodules. Takane [18] reported leucocytic infiltration in the myocardium of his animals fed thyroidine and iodine salt. Goodpasture [19] gave one group of rabbits thyroid gland and thyroxine; the animals showed characteristic clinical symptoms with definite, but relatively slight, myocardial lesions at autopsy. To a second group, similarly treated, he gave chloroform and found more striking, widespread myocardial necrosis. On the other hand, Rake and McEachern [20] recently failed to obtain any significant or specific changes in the myocardium of guinea pigs and rabbits "made hyperthyroid" by intramuscular administration of thyroxine. Other investigations, conducted chiefly for the effect of thyroid feeding on the size of the heart, report some degree of cardiac hypertrophy — Iscovesco [21] in rabbits, Hoskins [22] and Hewitt [23] in rats, Cameron and Carmichael [24] in rats and rabbits, Simonds and Brandes [25] in dogs.

Fahr concludes from his observations that the type of anatomical change in the heart indicates the action of a toxin circulating in the

blood stream; furthermore that this effect does not always occur, but when present is a condition of a true "Kropfherz" or goiter heart. Another opinion, of a theoretical nature, is that the increased metabolic processes and increased work demanded from the heart result in the myocardial damage. Or further, a view to which Goodpasture was inclined, hearts overstimulated by thyroid and laboring in a condition bordering on exhaustion are more susceptible to injury by toxic substances, *e.g.* from a mild terminal infection. This view, which has some experimental proof, does not preclude the possibility of a toxin of hyperthyroidism. Fahr found a "Kropfherz" in patients not only dying suddenly after operation and without evidence of terminal infection, but also dying with and without thymic enlargement. Furthermore, in some fatal cases, despite a terminal infection, the myocardium may be found normal at necropsy; in the hearts of four patients of one series,[12] no myocardial damage was evident, though bronchopneumonia had been a prominent factor in death.

In this question a comparison may be drawn with infectious disease. In diphtheria, for example, a toxin is always present; yet only in some instances may it act on the heart and kidneys to the extent that its effect is directly demonstrable or accompanied by anatomical changes.

Summary

In some cases of hyperthyroidism, certain degenerative and inflammatory changes that occur in the myocardium indicate a toxic origin and suggest the presence of a toxin circulating in the blood stream. Two cases showing such myocardial changes are reported.

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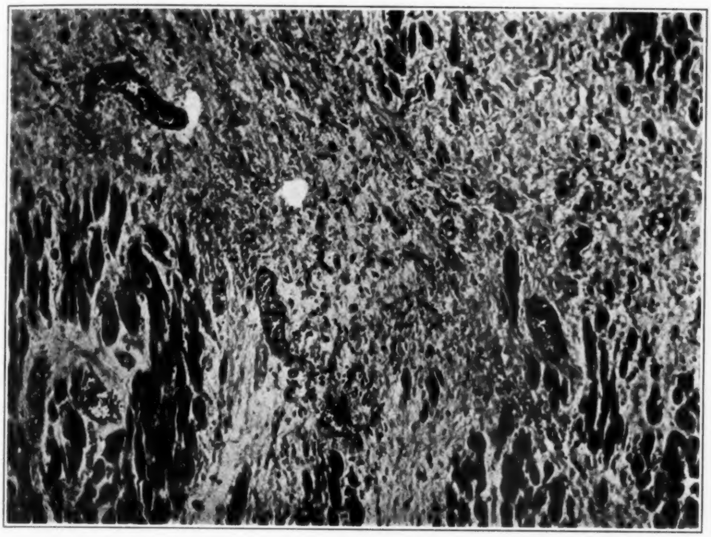
DESCRIPTION OF PLATE

Plate 39

Fig. 1. Case 1. Left ventricle. Focus of degeneration of myocardium; diffuse hemorrhage; diffuse lymphocytic infiltration; beginning fibrosis.

Fig. 2. Case 1. Left ventricle. Degeneration of myocardium; diffuse hemorrhage; diffuse lymphocytic infiltration; congestion of capillaries.



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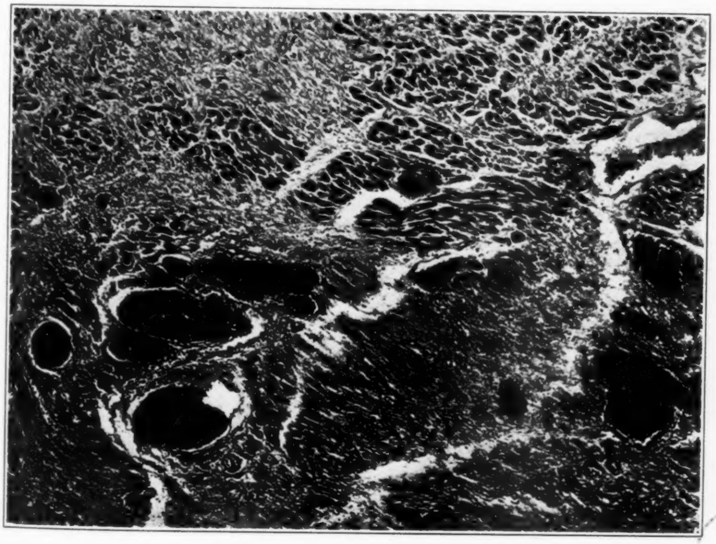

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